The Journal of Laboratory and Clinical Medicine

VOL. 26 APRIL, 1941 No. 7

CLINICAL AND EXPERIMENTAL

THE DIAGNOSTIC VALUE OF THE TAKATA-ARA REACTION IN THE CEREBROSPINAL FLUID*

PAUL B. SZANTO, M.D., AND SAMUEL BURACK, M.D., KANKAKEE, ILL.

LABORATORY tests devised for the examination of the cerebrospinal fluid are numerous. Despite this we agree with Kafka[1] that investigation of proteins in cerebrospinal fluid opens up new fields of research, particularly in the albumin-globulin relationship.

Eskuchen[2] stressed the importance of the different colloidal and protein reactions, the proteins (albumin and globulin) varying in their sensitivity to the various reagents. He introduced the term "protein spectrum." We must also consider the statement of Kafka[3] that pathologic processes result not only in quantitative change of the globulin and albumin and change of the ratio between both, but also qualitative alteration of the proteins themselves.

In 1925 Takata and Ara[4] introduced a new serum colloid test for differentiation between lobar and bronchopneumonia. In the same year they[5] used the test for examination of the cerebrospinal fluid to differentiate between bacterial meningitis and syphilis of the central nervous system.

The reaction is based on the following: If a solution of mercuric chloride is mixed with sodium carbonate in the presence of a protein colloidal system, for example, spinal fluid, there results a colloidal solution (HgO). The addition of fuchsin solution results in the formation of a violet blue color because the fuchsin is adsorbed by the colloid (HgO). In syphilitic spinal fluid there occurs a precipitation of the colloids with a resulting violet blue to blue color. Spinal fluid of bacterial meningitis gives a red color with little or no precipitation. Normal spinal fluid gives a violet blue color with no precipitation.

In 1929 Staub[6] and Jezler[7] introduced the Takata-Ara serum test for the diagnosis of parenchymatous liver diseases, especially for the diagnosis of liver

*From the Kankakee State Hospital, Kankakee. Dr. G. W. Morrow, Managing Officer.
Received for publication, March 4, 1940.

cirrhosis. In the following years many investigators (Staub,[8] Skouge,[9] Schindel and Barth,[10] Takata,[11] Kirk,[12] and Fulde[13]) affirmed the diagnostic value of this test for hepatic cirrhosis.

The reports of Takata and Ara on the cerebrospinal fluid in central nervous system syphilis were confirmed for the most part by various workers.

Münzer[14] examined 100 persons, of whom 30 had general paresis. These 30 gave the paretic type of Takata reaction, while the bacterial meningitis patients did not give consistent results. In those with paresis there was a parallelism between the Takata and Lange tests.

Nicole[15] found the Takata test more sensitive than the Ross-Jones, Weichbrodt, and Nonne-Apelt tests. He stated that the test was not specific for bacterial meningitis. Blum,[16] Cameron and McCulloch,[17] and Monias[18] reported similar findings.

Walton[19] examined 350 spinal fluids and found a parallelism between the Takata test and the Lange and mastic tests. He also stated the test was not specific for bacterial meningitis. All the workers mentioned followed more or less the original technique of Takata and Ara. Ucko[20] modified the Takata blood serum test, and this modification was applied to the spinal fluid by Fleischhacker.[21] Ornstein used the same modification, and both he and Fleischhacker affirmed the diagnostic value of the modified Takata-Ara reaction for neurosyphilis.

We felt it would be worth while to evaluate the results of the Takata-Ara test for the following reasons: First, we wished to prove the specificity of the test as originally described by Takata and Ara. Our material did not lend itself to evaluation of the specificity of the test for bacterial meningitis. Second, we wished to compare the diagnostic value of the test in relation to other routine cerebrospinal fluid determinations. Third, we wished to see which modification gave best results from the standpoint of both simplicity and sensitivity. Finally, we attempted to reach a better understanding of the mechanism behind the test.

TECHNIQUE AND INTERPRETATION

The Takata-Ara reaction can be performed in two ways: (1) the original five-tube method and (2) the so-called one-tube method. In the original technique, 1 c.c. of 0.3 per cent sodium chloride solution is added to four of five Wassermann test tubes. One cubic centimeter of spinal fluid is placed in the first and second tubes. The second tube is well agitated, and 1 c.c. of the mixture is added to the third tube, etc. One cubic centimeter from the fifth tube is discarded. One drop of 10 per cent sodium carbonate solution is added to each tube, followed by 0.3 c.c. of the Takata reagent. The Takata reagent consists of 0.5 per cent mercuric chloride and 0.02 per cent fuchsin solution in equal amounts. The tubes are well shaken and left at room temperature. The results are read after five, fifteen, and thirty minutes, and again after twenty-four hours. If the precipitation occurs immediately, or within a few minutes, we have a three-plus reaction; if the precipitation is complete after fifteen minutes, the reaction is two plus; after thirty minutes, one plus; and after twenty-four hours, one plus. The results were plotted on a curve. Spinal fluid from neurosyphilis gives complete precipitation in a short time.

In the one-tube method Ucko's modification was applied, wherein fuchsin solution is not used. The reagents required are 10 per cent anhydrous sodium carbonate and 0.5 per cent mercuric chloride solution. One cubic centimeter of spinal fluid is placed in a Wassermann tube to which is added one drop of the sodium carbonate solution and 0.1 c.c. of mercuric chloride solution. The exact amount of sodium carbonate used is very important because this substance influences the pH of the entire colloidal system. Glasoe and Sorum[22] showed that the sensitivity of a gold sol in the Lange spinal fluid test for paresis increases with an increase in the particle size, and decreases with an increase in the pH. We were able to show that the same principle applies to the Takata-Ara test. The results are read immediately, then after five, fifteen, thirty, sixty, and ninety minutes.

The interpretation of the results in the one-tube method is as follows: Three plus is indicated when flocculation occurs immediately with sedimentation. In a two-plus reaction complete flocculation and sedimentation occur in from five to thirty minutes. A one-plus reaction is indicated when an opacity occurs within thirty minutes. The reaction is negative when the mixture remains clear, or when there is a very slight opacity after thirty minutes.

At first we used both methods. However, we soon saw that our results with the one-tube method were as accurate as with the five-tube method.

MATERIAL AND RESULTS

We examined 314 persons. Of these there were 167 with general paresis, treated and untreated. Many of the treated patients had completely negative routine laboratory findings. In addition we had 147 individuals without paresis, 54 with dementia praecox, 40 with chronic alcoholism, 8 with idiopathic epilepsy, 9 with mental deficiency, 4 with psychoneurosis, 1 with pachymeningitis hemorrhagic interna, 1 with multiple sclerosis, 2 with manic-depressive psychosis, 15 with cerebral arteriosclerosis, 12 without psychosis, and 1 with traumatic psychosis.

GENERAL PARESIS

Our group of patients with general paresis had been on treatment for a number of years and some of them had resultant negative serologic and spinal fluid findings.

Of the 167 cases there were 34 with three-plus Takata reaction. In every case the spinal Wassermann was strongly positive. The Ross-Jones test gave a positive result in 19 cases, a trace in 10, and a negative result in 5. The cell count was less than ten in 11 cases, between ten and fifty in 14, and over fifty in 9. The gold sol reaction was in the first zone in 32 cases, and in the middle zone in 2.

There were 13 cases of two-plus Takata reaction. The spinal Wassermann was positive in every case. The Ross-Jones test was positive in 4, showed a trace in 7, and was negative in 2. The cell count was less than ten in 4 cases, between ten and fifty in 7, and over fifty in 2. The gold sol reaction was in the first zone in 7 cases, and in the middle zone in 6.

Sixty-seven persons gave a one-plus Takata reaction. The spinal Wassermann was positive in 64 and negative in 3. The Ross-Jones test was positive

in 2, showed a trace in 23, and was negative in 42. The cell count was less than ten in 49, between ten and fifty in 15, and over fifty in 3. The gold sol reaction was in the first zone in 1 case, in the middle zone in 32, in the end zone in 32, and negative in 2 cases.

There were 53 persons with negative Takata reaction. The spinal Wassermann was positive in 13 and negative in 40. The Ross-Jones test showed a trace in 2, and was negative in 51. The cell count was less than ten in 52 cases and between ten and fifty in 1. The gold sol reaction was in the first zone in 1 case and in the end zone in 3; there was none in the middle zone, and 49 cases were negative.

In the 147 persons without paresis the Takata reaction was negative.

The Takata-Ara reaction resembles the Weichbrodt test in that it utilizes mercuric chloride. However, there is a striking difference in the results. In the Weichbrodt test 0.3 c.c. of 1:2,000 mercuric chloride solution is added to 0.7 c.c. of spinal fluid in a test tube. A positive test is indicated by flocculation or opacity. We performed this test in 49 patients. In 31 both Takata-Ara and Weichbrodt tests were negative; in 18 the Takata-Ara reaction was positive and the Weichbrodt test was negative.

TABLE I

RELATIONSHIP OF TAKATA-ARA AND GOLD SOL REACTIONS TO THE GLOBULIN-ALBUMIN RATIO

CASE	SPINAL WASSERMANN	LANGE	CELL COUNT	ROSS-JONES	TAKATA	GLOBULIN / ALBUMIN
W. H.	++++	5555433111	62	Positive	+++	1.8
M. B.	++++	5444321100	65	Positive	+++	2.2
R. D.	++++	5555432111	66	Positive	+++	2.6
W. B.	++++	3342211100	42	Positive	++	1.4
G. W.	++++	5543311110	48	Positive	+++	1.9
V. M.	++++ in 1 c.c.; negative in 0.5 c.c.	2221110000	11	Negative	+	1.1
M. D.	++++	4332111000	6	Negative	+++	1.7
G. W.	++++	4332211000	15	Trace	++	1.5
E. F.	++++	5432111000	29	Trace	+++	2.8
T. D.	++++	5544432111	266	Positive	+++	2.4
T. M.	++++	5554331110	34	Trace	++	1.6
G. Y.	++++	3221110000	67	Trace	+	1.2
L. B.	++++	5433211100	28	Positive	+++	2.4
O. H.	++++	5543321110	40	Trace	+++	2.2
R. R.	++++	5554432110	21	Negative	+++	2.5
G. P.	++++	5554443210	36	Positive	+++	2.0

COMMENT

Our results indicate a striking parallelism between the Takata reaction and the gold sol reaction. Of the 114 persons with general paresis with positive Takata reaction the gold sol reaction was negative in only two. Of the 53 persons with general paresis with negative Takata reaction the gold sol reaction was positive in only 4. In addition, the degree of the Takata reaction shows a parallelism with the gold sol curve. The Takata reaction is much more sensitive than the Ross-Jones test. Of the 114 persons with positive Takata reaction the Ross-Jones test was negative in 49 cases, or 44 per cent. In regard to the cell count there is no parallelism.

MECHANISM

Takata and Ara believed that flocculation and sedimentation depend upon an alteration of the globulin-albumin ratio. We attempted to prove this theory and in addition to demonstrate that there is a parallelism between the globulin-albumin ratio in the spinal fluid and the Takata-Ara test.

The determination of the globulin-albumin ratio in the spinal fluid is very difficult because of the minimal amounts of protein present. We found that for this purpose Kafka's[1, 3, 23] volumetric method was best. We examined 16 persons with general paresis. The results are shown in Table I. If the globulin-albumin ratio was high, i.e., above 1.6, the Takata reaction was strongly positive; this was usually associated with a Lange curve in the first zone. On the other hand, with the ratio varying between 1.0 and 1.6, the Takata reaction was only weakly to moderately positive, and the Lange curve was usually in the middle zone. This seems to indicate that the higher the globulin-albumin ratio the stronger will be the Takata-Ara reaction.

SUMMARY

1. The Takata-Ara reaction is of great value in the diagnosis of syphilis of the central nervous system.

2. The Takata-Ara reaction shows an absolute parallelism to the gold sol reaction.

3. The Takata-Ara reaction is more sensitive than the Ross-Jones test.

4. The Takata-Ara reaction of the spinal fluid seems to depend upon the globulin-albumin ratio.

5. There appears to be a parallelism between the globulin-albumin ratio and the Takata-Ara reaction.

REFERENCES

1. Kafka, V.: Der Eiweissrelation der Liquor cerebrospinalis. I. Mitteilung, Ztschr. f. d. ges. Neurol. u. Psychiat. **106**: 54, 1926.
2. Eskuchen, K.: Lumbalpunction-Monograph, Berlin, 1919, Urban & Schwarzenberg.
3. Kafka, V., and Samson, K.: Die Eiweissrelation des Liquor cerebrospinalis. II. Mitteilung, Ztschr. f. d. ges. Neurol. u. Psychiat. **115**: 85, 1928.
4. Takata, M.: Ueber eine neue kolloidchemische Sero-Diagnostik der Lungentzuendung, Far East. A. Trop. Med. Tr. Sixth Congress **1**: 693, 1925.
5. Takata, M., and Ara, K.: Ueber eine neue kolloidchemische Liquorreaktion und ihre praktische Ergebnisse, Far East. A. Trop. Med. Tr. Sixth Congress **1**: 667, 1925.
6. Staub, H.: Ueber funktionelle Leberdiagnostik, Schweiz. med. Wchnschr. **59**: 308, 1929.
7. Jezler, A.: (a) Beitrag zur funktionellen Leberdiagnostik, Ztschr. f. klin. Med. **111**: 48, 1929; (b) Die Takata-Reaktion als differentialdiagnostisches Mittel bei der Untersuchung von Punktionsflüssigkeiten, insbesonders Aszites, Schweiz. med. Wchnschr. **60**: 52, 1930.
8. Staub, H.: Ueber beginnende Leberinsuffizienz, Deutsche med. Wchnschr. **57**: 2133, 1931.
9. Skouge, E.: Der Wert der Takata-Ara Reaktion und der Koagulationsband-bestimmung bei Leberkrankheiten, Klin. Wchnschr. **12**: 905, 1933.
10. Schindel, L., and Barth, E.: Die Bedeutung der Takata-Reaktion für die Diagnose der Lebererkrankungen in ihrem Verhältnis zur Galaktose- und Bilirubin-Belastung, Klin. Wchnschr. **13**: 1329, 1934; **13**: 1355, 1934.
11. Takata, M.: Ueber die Takata-Reaktion im Blut, Monograph, Kobe, 1935.
12. Kirk, R. C.: The Takata-Ara Test and Its Relation to Cirrhosis of the Liver, J. A. M. A. **107**: 1354, 1936.
13. Fulde, W.: Wert und Methodik verschiedener Leberfunktionsprüfungen für Klinik und Praxis, Klin. Wchnschr. **14**: 1201, 1935.
14. Münzer, F. T.: Ueber Liquorreaktion von Takata-Ara, Ztschr. f. d. ges. Neurol. u. Psychiat. **106**: 573, 1926.

15. Nicole, R.: Die Takata-Ara Reaktion in Liquor Cerebrospinalis, Ztschr. f. klin. Med. **106**: 192, 1927.
16. Blum, E.: Nachprüfung der kolloidchemischen Liquorreaktion von Takata-Ara an 200 Fällen, Ztschr. f. d. ges. Neurol. u. Psychiat. **110**: 504, 1927.
17. Cameron, D. E., and McCulloch, R.: Takata-Ara Test for Cerebrospinal Fluid, Canad. M. A. J. **23**: 557, 1930.
18. Monias, B. L.: Clinical Value of Spinal Fluid Test of Takata and Ara, J. LAB. & CLIN. MED. **14**: 67, 1928.
19. Walton, S. T.: A Comparative Study of 350 Spinal Fluid Examinations With Takata-Ara Test, J. LAB. & CLIN. MED. **16**: 1125, 1931.
20. Ucko, H.: (a) Serum Test for Diagnosis of Liver Disturbances, Guy's Hosp. Rep. **86**: 166, 1936; (b) Réaction de précipitation par le mercure dans le serum des hepatiques, Compt. rend. Soc. de biol. **118**: 534, 1935.
21. Fleischhacker, H. H.: The Takata Reaction on the Cerebrospinal Fluid in Syphilis of the Nervous System, J. Ment. Sc. **84**: 378, 1938.
22. Glasoe, P. U., and Sorum, C. M.: The Lange Test, J. LAB. & CLIN. MED. **25**: 1, 1939.
23. (1) Kafka, V., and Samson, K.: Die Eiweissrelation des Liquor cerebrospinalis, (a) III. Mitteilung, Ztschr. f. d. ges. Neurol. u. Psychiat. **117**: 128, 1928; (b) IV. Mitteilung, Ibid. **119**: 153, 1929; (c) V. Mitteilung, Ibid. **120**: 744, 1929. (2) Kafka, V.: Die Cerebrospinal Flussigkeit, Leipzig and Vienna, 1930, F. Deuticke.

100 EAST JEFFERY STREET

EPINEPHRINE SECRETION FROM THE ADRENAL GLANDS IN RELATION TO PARATHYROID ACTIVITY*

J. M. ROGOFF, M.D., SC.D., AND GEORGE N. STEWART, M.S., PITTSBURGH, PA., AND RUTH CORTELL, PH.D., CHICAGO, ILL.

IN AN earlier study[1] it was shown that the rate of liberation of epinephrine from the adrenal glands in dogs with experimental hyperthyroidism is not different from that in normal, control animals. Experiments on hypothyroidism, induced by extirpation of the thyroids, may be complicated by possible effects of concurrent hypoparathyroidism. Experiments on thyroparathyroidectomized animals, therefore, have been included in this report.

Probability of functional interrelationship between the parathyroids and the adrenals is suggested from certain earlier observations.[2] The evidence indicates that such an interrelationship is probably concerned with the function of the adrenal cortex, but it does not exclude the possibility that the medulla may play a significant role in the phenomena observed.

Rogoff and Stewart[3] found that adrenalectomized dogs develop symptoms that are commonly associated with disturbances in parathyroid function. Muscular twitching, spasms, and tetanic convulsions often occur. Furthermore, bilateral adrenalectomy is commonly followed by an increase in calcium content of the blood.[4] At autopsy severe congestion and hemorrhages are found in the alimentary canal of adrenalectomized dogs, similar to that observed by Collip[5] in unoperated dogs following administration of large amounts of parathyroid extract.

*From the Physiological Laboratory, University of Chicago, and the Laboratory of Experimental Endocrinology,† School of Medicine, University of Pittsburgh.

†Supported by the G. N. Stewart Memorial Fund, founded by contributions from the Louis D. Beaumont Trust, N. L. Dauby, Mrs. Frances W. Lang, and Howard E. Wise.

Received for publication, March 5, 1940.

The changes that occur in the blood and the disturbances in the alimentary canal that are found during acute adrenal insufficiency in adrenalectomized dogs have been observed in animals with subacute and chronic adrenal insufficiency created by the method described by Rogoff.[6] In these animals, as well as in those surviving bilateral adrenalectomy without treatment nearly or up to a fortnight, it has been observed that there is a relatively high incidence of parathyroid enlargement.[2] Some of the blood changes that occur in these animals are the same as those caused by administration of parathyroid extract.

Certain changes that occur in the rat incisor following bilateral adrenalectomy were found by Schour and Rogoff[7] to be similar to the disturbances in calcification of the dentine observed by Schour, Tweedy, and McJunkin[8] following administration of parathyroid extract. The rat incisor as an indicator for changes in calcium metabolism is suggested from the sensitive response to parathyroidectomy observed by Erdheim.[9]

The foregoing experimental evidence points to a probable adrenal-parathyroid interrelationship. It appears that the adrenal cortex is concerned with this possible interrelationship. Experimental data concerning the adrenal medulla in relation to parathyroid function is less convincing. Some observations on the pharmacodynamics of epinephrine suggest a possible influence on calcium metabolism. However, unless such observations are quantitatively related to amounts of epinephrine that the adrenals are known to be capable of liberating, they may be misleading. Mathieu and Bacq[10] failed to obtain a significant effect on the blood calcium of dogs with parathyroid insufficiency when they administered physiologic quantities of epinephrine.

Some investigators have found a reduction in the epinephrine content of the adrenals in animals with parathyroid insufficiency,[11, 12] while others observed no change.[13-15] Of course, the amount of epinephrine stored in the adrenal cannot indicate the rate of its liberation; it can represent no more than the balance between production and liberation. However, these conflicting results suggest the possibility that thyroparathyroidectomy sometimes may lead to disturbances which interfere with storage of epinephrine in the adrenals. These disturbances may not develop in all thyroparathyroidectomized animals. This suggestion might be offered in explanation of similar observations regarding the rate of epinephrine secretion reported in this article.

Indirect evidence has been offered in support of the view that certain manifestations of parathyroid insufficiency are associated with a corresponding increase in circulating epinephrine.[16] Czarnecki and Sarabia[17] found no evidence of increased liberation of epinephrine from the adrenals in dogs with parathyroid insufficiency, and Georgopolous obtained negative results with peripheral blood of rabbits. These investigators did not make quantitative determinations of the rate of output of epinephrine from the adrenals.

Our investigation is concerned with the question whether experimental hypoparathyroidism or hyperparathyroidism is associated with a detectable change in the rate of liberation of epinephrine from the adrenal glands. Hyperparathyroidism was induced by administration of parathyroid extract,* and

*Generously supplied by Eli Lilly and Company.

hypoparathyroidism by thyroparathyroidectomy which, of course, includes any possible influence that might result from loss of the thyroid.

The characteristic manifestations of hypothyroidism are not readily obtained in adult dogs following thyroidectomy. This is generally attributed to the presence of accessory thyroid tissue or to incomplete removal of the thyroids. However, definite manifestations of hypothyroidism can be induced by complete thyroidectomy in young dogs if sufficient parathyroid tissue remains to prevent the complications of parathyroid deficiency. It may be assumed also that complete thyroidectomy in older dogs might be associated with some physiologic disturbances that are not as yet recognized.

TABLE I

EPINEPHRINE OUTPUT IN THYROPARATHYROIDECTOMIZED DOGS

RECORD NUMBER	SEX	BODY WEIGHT (KG.)		SERUM CALCIUM PER 100 C.C. (MG.)		ADRENAL WEIGHT (GM.)		EPINEPHRINE OUTPUT PER MINUTE (MG.)	
		INITIAL	FINAL	INITIAL	FINAL	RIGHT	LEFT	PER ANIMAL	PER KG.
21	M	11.7	10.1			0.73	0.76	0.00016	0.000016
38	M	12.0	12.0	10.17	6.10	0.52	0.57	0.0021	0.00017
61	M	8.8	8.5	11.85	7.44	0.40	0.40	0.00111	0.00013
62	M	7.3	6.3	12.91	4.39	0.41	0.38	0.00565	0.0009
68	F	9.8	8.8	10.02	4.03	0.64	0.64	0.0004	0.000045
69	M	10.5	10.7	10.60	5.55	0.62	0.59	0.00225	0.00021
71	M	7.3	7.5	11.66	5.28	0.49	0.50	0.00135	0.00018
73	M	13.0	11.9	12.27	4.88	0.70	0.70	0.0024	0.0002
74	F	8.0	7.6	10.88	5.76	0.56	0.56	0.00023	0.00003
2-15	M	12.1	11.5			0.78	0.76	0.0017	0.00015
1-81	M	13.3	13.5			0.85	0.76	0.0023	0.00017

TABLE II

EPINEPHRINE OUTPUT IN THYROPARATHYROIDECTOMIZED CATS

RECORD NUMBER	SEX	BODY WEIGHT (KG.)		DURATION (DAYS)	SYMPTOMS	ADRENAL WEIGHT (GM.)		EPINEPHRINE OUTPUT PER MINUTE (MG.)	
		INITIAL	FINAL			RIGHT	LEFT	PER ANIMAL	PER KG.
1-83	F	2.1	2.2	8	−	0.105	0.110	0.0004	0.00018
1-84	F	2.5	2.5	4	−	0.15	0.15	0.0002	0.00008
1-86	F	2.7	2.6	9	+−	0.21	0.20	0.00075	0.00029
1-96	F	1.9	1.9	11	−	0.08	0.09	0.00015	0.00008
2-22	M	4.54	3.9	14	+−	0.28	0.30	0.0011	0.00028
2-31	F	2.61	2.04	16	++	0.26	0.26	0.0002	0.0001
2-32	F-	1.93	1.7	2	+++	0.15	0.18	0.0005	0.00029
2-33	F	3.64	3.3	2	++	0.33	0.33	0.00033	0.0001
2-34	F	2.73	2.27	6	+++	0.21	0.21	0.00025	0.00011
2-40	M	2.94	2.32	27	+−	0.14	0.12	0.0005	0.00022
2-41	F	3.18	3.63	50	+−	0.19	0.21	0.00025	0.00007

THYROPARATHYROIDECTOMY

Thyroparathyroidectomy was performed on 11 dogs (Table I) and 11 cats (Table II). Quantitative determination of the rate of liberation of epinephrine from the adrenals was made by the method of Stewart and Rogoff.[18] The epinephrine output from the adrenals was determined at periods ranging between two and thirty days after thyroparathyroidectomy in the dogs, and between two and fifty days in the cats.

In the cat (2-41) on which determination of the epinephrine output was made fifty days after thyroparathyroidectomy, moderate symptoms were present during about half of the experimental period. The symptoms then gradually subsided. One cat (2-33) was thyroparathyroidectomized during advanced pregnancy. Marked tetany developed on the second day after the operation, indicating that the parathyroids of the fetuses did not protect the mother. That fetal endocrine organs do not function in place of the corresponding maternal organs when extirpated was found in the case of the adrenal (Stewart and Rogoff) and in recent observations (unpublished) in case of the pancreas.

Manifestations of parathyroid deficiency, ranging from apathy and mild muscular twitchings to severe convulsive seizures, were present in nearly all animals at the time when they were sacrificed for determination of the epinephrine output. In 8 of the dogs total calcium was determined in the blood serum by the Clark and Collip modification of the Kramer-Tisdall method.[19] As shown in Table I, a decided reduction in serum calcium occurred in all animals. Some of the dogs received parathyroid extract or calcium therapy to control severe symptoms. In these cases the therapy was discontinued to permit the symptoms to reappear before the animal was used for determination of the epinephrine output. In the cats and in four of the dogs no therapeutic measures were employed.

Dog 21 developed symptoms of parathyroid insufficiency eight days after thyroparathyroidectomy. Calcium lactate (10 Gm. daily) was administered for eighteen days thereafter and was discontinued four days prior to determination of the epinephrine output. The symptoms were controlled by the calcium therapy and reappeared when therapy was discontinued. Clonic convulsions were present when the animal was sacrificed for determination of the epinephrine output.

In Dog 69 tetany developed on the fourth day after thyroparathyroidectomy. Calcium gluconate (10 c.c. of 10 per cent solution), which controlled the symptoms, was administered intravenously. Three days later the symptoms reappeared and the animal was sacrificed the following day for determination of the epinephrine output, without receiving any further treatment.

Dogs 61 and 62, both young, developed tetany on the second day following thyroparathyroidectomy. Each dog received 50 units of parathyroid extract. The symptoms were controlled, and Dog 61 was sacrificed the following day for determination of the epinephrine output. Dog 62 developed violent convulsions on the fifth day, and 30 units of parathyroid extract was administered. Determination of the epinephrine output was made the following day.

Dog 71 had tetany on the second day after thyroparathyroidectomy. He received 100 units of parathyroid extract and the symptoms were relieved. Four days later, when the symptoms were again in evidence, the epinephrine output from the adrenals was determined.

Dogs 38 and 74 developed severe tetany on the third and second day, respectively, following thyroparathyroidectomy. Determination of the epinephrine output from the adrenals was then made, without resorting to therapeutic measures.

Dog 68 developed mild tetany on the fourth day and it was present on the sixth day when determination of the epinephrine output was made. Mild tetany developed in Dog 73 on the fifth day following thyroparathyroidectomy, when the animal was sacrificed for determination of the epinephrine output from the adrenals.

HYPERPARATHYROIDISM

Ten dogs were subjected to the influence of parathyroid extract for periods ranging between one and fourteen days (Table III). In all cases a marked

elevation of the level of total calcium in the blood existed at the time when the animals were sacrificed for determination of the epinephrine output from the adrenals.

TABLE III

EPINEPHRINE OUTPUT IN DOGS WITH EXPERIMENTAL HYPERPARATHYROIDISM

RECORD NUMBER	SEX	BODY WEIGHT (KG.)		SERUM CALCIUM PER 100 C.C. (MG.)		COMBINED WEIGHT OF THYROIDS (GM.)	ADRENAL WEIGHT (GM.)		EPINEPHRINE OUTPUT PER MINUTE (MG.)	
		INITIAL	FINAL	INITIAL	FINAL		RIGHT	LEFT	PER ANIMAL	PER KG.
41	M	8.6	8.4	9.56	14.74		0.41	0.43	0.00227	0.00027
43	M	6.8	6.0	9.84	15.83		0.44	0.39	0.00024	0.00004
44	M	8.6	7.7	11.44	15.37		0.73	0.63	0.00078	0.0001
47	F	5.9	5.5	11.03	16.66	1.21	0.40	0.40	0.00067	0.00012
48	F	5.4	4.3	11.05	16.63	0.44	0.38	0.38	0.00146	0.00034
50	M	10.8	10.6	10.43	10.54	1.08	0.70	0.78	0.00233	0.00022
55	M	9.1	7.7	9.88	15.29	1.74	0.43	0.49	0.00185	0.00024
56	M	11.0	9.9	10.69	18.38	1.14	0.63	0.54	0.00109	0.00011
57	M	10.7	9.7	9.31	11.71	1.13	0.82	0.65	0.00262	0.00027
60	M	7.8	7.3	10.25	19.03	0.90	0.70	0.70	0.00204	0.00028

Dog 41 received 300 units of parathyroid extract in the morning and 200 units in the afternoon of the same day. Epinephrine output was determined eighteen hours after the last injection of parathyroid extract.

Dog 43 received 220 units of parathyroid extract on the first day, 250 units the next day, and 100 units on the third day. Epinephrine output was determined eighteen hours after the last injection of parathyroid extract.

Dog 44 received 200 units of parathyroid extract in the morning and 200 units in the afternoon of the same day. Epinephrine output was determined seventeen hours after the last injection of parathyroid extract.

Dog 47 received a daily injection of 100 units of parathyroid extract on each of three successive days. Epinephrine output was determined on the fourth day. Parathyroid extract caused considerable vomiting. Severe congestion and hemorrhage in the alimentary canal were found at autopsy.

Dog 48 received 50, 30, 30, 50, and 30 units of parathyroid extract on five consecutive days, respectively. Epinephrine output was determined on the sixth day.

Dog 50 received 50, 50, 50, 100, 100, and 100 units of parathyroid extract on six consecutive days, respectively. Epinephrine output was determined forty-eight hours after the last injection of extract.

Dog 55 received 50 units of parathyroid extract daily except on the sixth and twelfth days. On the thirteenth day 75 units were administered and the epinephrine output was determined on the fourteenth day.

Dog 56 received 50 units of parathyroid extract daily for twelve consecutive days. Epinephrine output was determined on the thirteenth day.

Dog 57 received 50 units of parathyroid extract daily for five consecutive days and 20 units on the sixth day. Epinephrine output was determined forty-eight hours after the last injection of parathyroid extract. A hemorrhagic ulcer was found in the stomach at autopsy. In this animal and in Dog 50 the serum calcium had returned to normal level when epinephrine output was determined.

Dog 60 received two injections of 50 units each of parathyroid extract, seven hours apart, on one day, and 50 units the next day; no parathyroid extract was administered the following four days; then 5 doses of 50 units each were administered during the next thirty hours. Epinephrine output was determined fifteen hours after the last injection of parathyroid extract.

DISCUSSION

In only one animal receiving parathyroid extract (Dog 43) was the rate of liberation of epinephrine from the adrenals found to be outside the range ordinarily occurring in normal animals. In view of the normal output found in the other nine animals, we hesitate to attach significance to the relatively low epinephrine output found in this animal.

None of the thyroparathyroidectomized cats showed significant alteration in the rate of liberation of epinephrine from the adrenals. Among the dogs, however, we found a definite reduction in epinephrine output in three of the eleven experimental animals. Normal rate of liberation was found in the other eight dogs. It appears probable that in some thyroparathyroidectomized dogs conditions may develop which can affect the adrenal medulla. This possibility might explain the conflicting results that have been reported concerning the epinephrine store in the adrenals, previously mentioned.

It is known that existence of accessory parathyroids is not uncommon. The suggestion may be made that such accessories, though functionally inadequate to prevent the usual manifestations of parathyroid deficiency in thyroparathyroidectomized animals, might suffice to prevent reduction of the epinephrine output. This could explain the negative results in the cats and in the 8 dogs that showed normal epinephrine output.

The possible influence of relative deficiency of thyroid, together with parathyroid deficiency occurring in some animals but not in others, may be suggested. In the light of present knowledge, however, this can be no more than mere speculation.

The results of our experiments, while not conclusive, support the existing suggestion of a probable functional interrelationship between the parathyroids and the adrenal medulla. Further investigation is necessary to obtain more unequivocal evidence. However, it appears that under some conditions, the exact nature of which is not known, parathyroid insufficiency can lead to a reduced rate of liberation of epinephrine from the adrenal glands.

SUMMARY

1. Under certain conditions, the nature of which is obscure, parathyroid insufficiency can lead to reduced epinephrine output from the adrenals.

2. Hyperparathyroidism, induced by administration of parathyroid extract, does not appear to influence the rate of epinephrine secretion from the adrenals.

REFERENCES

1. Rogoff, J. M., and Cortell, R.: Epinephrine Output From the Adrenal Glands in Experimental Hyperthyroidism, J. LAB. & CLIN. MED. **26**: 981, 1941.
2. Rogoff, J. M.: Observations on Functional Interrelationship Between the Adrenal and Parathyroid Glands, Science **80**: 319, 1934.
3. Rogoff, J. M., and Stewart, G. N.: Studies on Adrenal Insufficiency in Dogs. I. Control Animals Not Subjected to Any Treatment, Am. J. Physiol. **78**: 683, 1926.
4. Rogoff, J. M., and Stewart, G. N.: Studies on Adrenal Insufficiency. VII. Further Blood Studies (Cholesterol and Calcium) in Control Adrenalectomized Dogs, Am. J. Physiol. **86**: 25, 1928.
5. Collip, J. B.: The Production of Some of the Phenomena Peculiar to Parathyroid Overdosage in Dogs by Means of Certain Inorganic Salts, Am. J. Physiol. **76**: 472, 1926.
6. Rogoff, J. M.: Experimental Production of Chronic and Subacute Adrenal Insufficiency in Dogs and Cats, Proc. Soc. Exper. Biol. & Med. **24**: 1240, 1932.

7. Schour, I., and Rogoff, J. M.: The Influence of the Adrenal Glands on Calcium Metabolism, Science **83**: 267, 1936; Changes in the Rat Incisor Following Bilateral Adrenalectomy, Am. J. Physiol. **115**: 334, 1936.
8. Schour, I., Tweedy, W. R., and McJunkin, F. A.: The Effect of Single and Multiple Doses of the Parathyroid Hormone on the Calcification of the Dentine of the Rat Incisor, Am. J. Path. **10**: 321, 1934.
9. Erdheim, J.: Tetania parathyreopriva, Mitt. a. d. Grenzgeb. d. Med. u. Chir. **16**: 632, 1906.
10. Mathieu, F., and Bacq, Z. M.: Action of Epinephrine in Dog With Parathyroid Insufficiency, Arch. internat. de physiol. **38**: 160, 1934.
11. Herring, P. T.: The Effects of Thyroidectomy and Thyroid-Feeding Upon the Adrenalin Content of the Suprarenals, Quart. J. Exper. Physiol. **9**: 391, 1916.
12. Binet, L., and Weller, G.: La teneur des glandes surrénales en adrénaline chez les animaux parathyroprives, Compt. rend. Soc. de biol. **114**: 985, 1933.
13. Gley, E.: Contribution a l'etude des interrelations humorales. II. Valeur physiologique de la glande surrenale des animaux ethyroides, Arch. internat. de physiol. **14**: 175, 1914.
14. Bacq, Z. M., and Mathieu, F.: Teneur des glandes surrénales en adrénaline chez le chien en tétanie latente, Arch. internat. de physiol. **42**: 279, 1935.
15. Georgopolous, M.: Beziehungen zwischen Parathyroidea und Chromaffinen System, Ztschr. f. klin. Med. **75**: 411, 1912.
16. Falta, W., and Rudinger, C.: Klinische und experimentelle Studien über Tetanie, Verhandl. d. 26 Kong. f. inn. Med., p. 405, 1909.
17. Czarnecki, E., and Sarabia, L.: La Secretion surrenale d'adrenaline chez les Chiens ethyroides, Compt. rend. Soc. de biol. **97**: 455, 1927.
18. Stewart, G. N., and Rogoff, J. M.: The Average Epinephrine Output in Cats and Dogs, Am. J. Physiol. **66**: 235, 1923.
19. Clark, E. P., and Collip, J. B.: A Study of the Tisdall Method for the Determination of Blood Serum Calcium With a Suggested Modification, J. Biol. Chem. **63**: 461, 1925.

A CASE OF INSULIN ALLERGY SIMULATING CORONARY OCCLUSION*

HARRY F. WECHSLER, M.D., LAURENCE FARMER, M.D., AND JEROME A. URBAN, M.D., NEW YORK, N. Y.

NUMEROUS cases of insulin allergy have been reported in the literature. In the majority of these cases, local or generalized urticaria and angioneurotic edema dominated the clinical picture. In a smaller number of instances, there were more severe allergic reactions, such as circulatory impairment, gastrointestinal symptoms, pulmonary edema, and collapse. Immunologic studies revealed that the observed hypersensitivity was due either to protein impurities accompanying the insulin or to pure crystalline insulin itself. Passive transfer of the sensitivity was effected in several instances. A comprehensive review of the literature has recently been published by Herzstein and Pollack.[1]

The following case of insulin allergy, which followed the general immunologic pattern, presented an interesting, and as far as we know, a not yet described differential diagnostic problem.

A. J., a 65-year-old white woman, was admitted to the Lenox Hill Hospital for the first time on January 6, 1932, for the reduction and immobilization of a fracture of the left radius. She was found to be suffering from diabetes mellitus and was put on a diet and 20 units of insulin (Iletin) daily. She discontinued her diet after one year, but continued to take the 20 units of Iletin.

*From the Medical Service of Dr. Otto M. Schwerdtfeger and the Allergy Clinic of Dr. Laurence Farmer, Lenox Hill Hospital, New York.
Received for publication, March 13, 1940.

She was readmitted to the hospital on July 21, 1936, for an osteomyelitis of the right toe. During her stay at the hospital she was again put on a diet and 20 units of Iletin. She was discharged on September 21, 1936, and told to discontinue her insulin as the diet alone sufficed to control the diabetes. The patient took no insulin until December, 1938, when 5 units were administered because of glycosuria and pruritus vulvae. Ten minutes after this injection she became unconscious, dyspneic, thrashed about, and had a cold sweat. She was given orange juice and completely recovered from the attack in about two hours. Since this episode she has been fearful of taking insulin.

Her third admission to the Lenox Hill Hospital was on April 3, 1939, for an ulcer of the right foot. Physical examination revealed a markedly obese elderly woman who did not appear acutely ill. Her temperature was 100.8° F.; her respirations were 20 per minute. There was no dyspnea or cyanosis. Numerous areas of vitiligo were present in the skin, and on the sole of the right foot was an ulcerated callus.

The pupils were equal, regular, and reacted to light and accommodation. The sclerae were clear. There was no exophthalmos, lagophthalmos, or nystagmus. The skin of the eyelids was loose, redundant, and had a puffy appearance. The conjunctivae were injected.

The mouth was edentulous. The tongue was slightly coated. The tonsils were small and cryptic, and the pharynx was congested.

The lungs were resonant throughout with normal breath sounds. No râles were audible.

The point of maximal impulse of the apex was in the fourth interspace in the midclavicular line. The border could not be accurately elicited because of obesity. The second aortic sound was accentuated and somewhat harsh. A systolic murmur was heard over the aortic area and a soft, low-pitched systolic murmur at the apex. The pulses were equal, regular, and the rate was 88. The blood pressure was 170 systolic and 90 diastolic.

No organs or masses were palpable in the abdomen. There was slight ankle and pretibial edema. The dorsalis pedis arterial pulsations were normal bilaterally. The reflexes were equal and active. There were no pathologic reflexes.

COURSE

The fasting blood sugar was 210 mg. per cent, and the urine revealed 1.4 per cent sugar and a trace of acetone. On April 5, 1939, the patient was given 20 units of Iletin in the morning after breakfast. Forty-five minutes after the injection the patient suddenly went into collapse. She complained of dizziness, vomited her breakfast, and was very dyspneic. There was no precordial pain. The skin was pale, cold, and clammy. The heart sounds were feeble and tic-tac in quality. The pulse was imperceptible at the wrist, and the blood pressure could not be obtained. Her venous pressure was 124 mm. The white blood count was 16,000; polymorphonuclear cells 81 per cent, twelve of which were immature; lymphocytes 18 per cent; monocytes 1 per cent.

An electrocardiogram taken fifteen minutes after the onset of the attack showed only minimal changes. These consisted principally of a depression of the S-T interval in Lead I, deep S and an inversion of the T-wave in Lead III, and a small R-wave and flattened T-wave in several of the precordial leads.

The patient was given 200 c.c. of orange juice and one-sixth grain of morphine, and was placed in an oxygen tent. She recovered rapidly and progressively from her shock; twelve hours later her blood pressure was 150 systolic and 100 diastolic; her heart sounds were of good quality and her pulse rate was 84.

Although the cardiologist considered various other possibilities, because of the white count of 16,000, the electrocardiographic changes, the fall in the blood pressure, and the tic-tac heart sounds, he favored the diagnosis of coronary occlusion and advised treating the patient for such. The features which militated against that diagnosis were the rapid recovery from the profound shock, the absence of temperature, and the normal erythrocyte sedimentation rate. In addition, a history was subsequently obtained of a similar attack three years previously following the injection of insulin.

During the next few days her blood pressure fluctuated between 90 systolic and 60 diastolic to 150 systolic and 90 diastolic. On April 6, the fasting blood sugar was 192 mg. per cent. The urine remained sugar free on diet alone, with only occasional slight spilling. The temperature remained normal throughout her stay. There was no increase in the erythrocyte sedimentation rate. Electrocardiograms taken at frequent intervals showed slight and inconclusive changes. These were practically confined to the T-wave in Lead III. The S-T interval returned to the base line the day after the attack.

On May 17, 1939, six weeks after her attack, she was given three units of Iletin after breakfast. Her fasting blood sugar was 132 mg. per cent. Twenty-five minutes later she went into a profound shock. Fifteen minutes after the beginning of this attack the blood sugar was 143 mg. per cent.

Twenty minutes after the beginning of the attack the pulse was barely perceptible and very rapid. The blood pressure was 65 systolic and 0 diastolic. The patient was cyanotic, dyspneic, and exhibited irregular sighing respirations. Her skin was cold, moist, and clammy. She showed tremors and convulsive movements. Adrenalin injected subcutaneously gave her slight relief; she was drowsy for several hours. The blood pressure six hours later was 85 systolic and 65 diastolic. The white blood cell count eight hours later was 9,200. The electrocardiogram shortly after the onset of the attack showed minimal changes. An allergic work-up was done one week later. Table I contains the skin tests.

The patient gave positive reactions to all tested brands of insulin, including crystalline insulin. She did not react to pork, beef, and lamb extracts. These tests show clearly that she was allergic to pure crystalline insulin. Passive transfer was attempted. The result was inconclusive as the normal test person gave positive intradermal reactions also in untreated skin areas.

TABLE I

TEST SUBSTANCE	METHOD OF TESTING		
	SCRATCH	PUNCTURE	INTRADERMAL
Lilly's Iletin 40 U.	+++	+++	
Lilly's Iletin special (Beef) 20 U.	+++	+++	
Squibb's insulin 20 U.	±	+ (+)	
Stearn's insulin	±	+	
Lilly's Iletin zinc 40 U.	++	+ (+)	
Stearn's zinc insulin	++	++	
Lilly's protamine zinc insulin 40 U.	++ (+)	++	
Pork	0	0	0
Beef	0	0	0
Lamb	0	0	0

SUMMARY

The injection of 20 units, and later of 3 units, of Iletin (Lilly) in a 65-year-old arteriosclerotic hypertensive diabetic patient gave rise to a syndrome strongly simulating coronary occlusion. Reviewing the case from the viewpoint of a possible allergy, it is clear that we are dealing with the symptoms of anaphylactic shock. Skin tests corroborated this assumption.

REFERENCE

1. Herzstein, Joseph, and Pollack, Herbert: J. Mt. Sinai Hosp. **6**: 3, 1939.

THE ROLE OF METALS IN CARBOHYDRATE METABOLISM*

ZOLTON T. WIRTSCHAFTER, B.S., M.D., CLEVELAND, OHIO

EXTENSIVE experimental and clinical investigations concerning the effect of various metals-insulin mixtures have been recorded by many workers. Thus, it is well known that the addition of 0.1 per cent of zinc to insulin delays and prolongs the action of the latter, while the addition of 0.9 per cent of zinc completely inhibits the insulin effect. This phenomenon brings up the question of the possible basic role of the metals in carbohydrate metabolism under both physiologic and pathologic conditions. To evaluate the state of our present knowledge the important contributions in this field are summarized.

Sodium.—Magenta[1] studied the effect of sodium fluoride on the glycemic levels of dogs. He found that the intravenous injection of sodium fluoride did not provoke a glycosuria or a hyperglycemia in them. His observations supplement those previously described by Goldenberg,[2] who observed a glycosuria among both lambs and horses subjected to prolonged ingestion of sodium fluoride.

In 1925 Abelin and Goldener[3] investigated the effect of certain sodium salts on the hypoglycemic action of insulin in animals. The oral administration of 2 Gm. of monosodium phosphate ($NaH_2PO_4.H_2O$) or 2 Gm. of a mixture of sodium chloride, sodium bicarbonate, and sodium sulfate produced a marked augmentation of the hypoglycemic effect of 4 to 6 units of insulin given simultaneously. The use of 2 Gm. of disodium phosphate ($Na_2HPO_4.12H_2O$) has no appreciable effect on insulin action (Abelin and Goldener[3]).

Apparently these authors did not understand nor question the reason why disodium phosphate did not equal or increase the hypoglycemic effect of insulin action. It is clear that they did not take into consideration the twelve times larger amount of water crystallization in the disodium phosphate molecule, thus producing a unit containing much less sodium per gram than monosodium phosphate. It is quite probable that if this fact were taken into account, the effect of the disodium salt would be equal to or greater than that of the monosodium phosphate. The fact that the effect of the sodium salt mixture was equal to that of the monosodium phosphate appears to indicate that the active portion of the molecule is the sodium ion. Abelin and Goldener[3] did not determine whether the hypoglycemic effect of the sodium salts is a direct effect on carbohydrate metabolism or merely an indirect result of their effect on insulin.

This problem has been further investigated by McQuarrie, Thompson, and Anderson,[4] who demonstrated that the hypoglycemic effect of sodium salts is a direct one on carbohydrate metabolism, since a drop in blood sugar level of persons with diabetes could be produced without the simultaneous injection of

*From the Department of Medicine, City Hospital, Western Reserve University, and the Department of Medicine, Mt. Sinai Hospital, Cleveland.

Received for publication, March 11, 1940.

insulin. They also studied the effect of excessive ingestion of sodium salts on the carbohydrate metabolism of four diabetic children, between 13 and 15 years of age. In each case it was found that the ingestion of approximately 60 Gm. of sodium chloride daily produced a marked decrease in the amount of glucose appearing in the urine. In the case of a 15-year-old boy who required 32 units of insulin daily, the administration of 60 Gm. of salt decreased the urinary glucose from 73 Gm. to a minimum of 13 Gm. after two days. After seven days the salt was discontinued and the urinary glucose returned to the original level within three days. Although the fasting blood sugar level was decreased and the respiratory quotient was increased by salt administration, the appearance of the sugar tolerance curve did not show any change. Less insulin was required during the period of salt administration, since typical insulin reactions occurred when the insulin dosage was not decreased. It was shown that the effect was due to the sodium ion, since the use of other sodium salts produced similar effects while other chlorides did not. The administration of excessive amounts of sodium chloride did not produce hypoglycemia in a normal control subject. The mechanism of the sodium action is not known. It does not appear that the phenomenon can be satisfactorily explained solely on the basis of water and glucose retention in the tissues. McQuarrie and associates[4] investigated the possible importance of cortical extract in explanation of the sodium effect; e.g., after producing a marked decrease in the glycosuria of a person with severe diabetes, these workers substituted adrenal cortical extract for the sodium chloride and found that the degree of glycosuria returned toward the presaline level.

Potassium.—The action of the potassium ion on carbohydrate metabolism is, in general, directly opposed to that of the sodium ion. Potassium chloride, when administered alone, tends to decrease carbohydrate tolerance and to raise the blood sugar level, both in man and experimental animals. As in the case of sodium, the exact mechanism involved is not definitely known. Rathery, Bertoliatti,[5] and others found that the blood potassium level of diabetic individuals is characteristically elevated. McQuarrie[4] noted a drop in serum potassium of diabetic persons while on a high sodium chloride intake. The high vegetable diet of the average diabetic person is high in potassium content. McQuarrie[4] and his co-workers have reported an increase averaging 50 per cent in the excretion of glucose in persons with diabetes while being fed extra amounts of potassium chloride. While receiving 10 to 20 Gm. of potassium chloride, a low sodium diet, and the carbohydrate intake kept constant, the urine glucose excretion increased from 60 to 70 Gm. to 70 to 100 Gm. daily. They found that the oral administration of potassium chloride to a diabetic patient receiving large amounts of sodium chloride resulted in an elevation of the glucose excretion. The results appeared to indicate that the administration of one milliequivalent of potassium neutralized the effect of three milliequivalents of sodium.

The antagonistic effects of sodium and potassium were similar in diabetic persons receiving insulin and those not receiving insulin therapy. McQuarrie and co-workers[4] report the relief of symptoms due to hypoglycemia in an acromegalic person by the administration of potassium chloride.

Calcium-Magnesium.—Kiyohara, Morita, and Muta[6] investigated the effect of potassium on the glycemic levels of dogs by means of perfusion of the isolated pancreas with blood to which potassium chloride had been added. The addition

of potassium chloride (0.1 Gm. per 100 c.c. of blood) modified the content of the blood sugar. Kiyohara, Morita, and Muta[6] stated that the potassium ion did not exercise any direct influence on the insulinar tissue of the pancreas. Their work clearly showed that in rabbits an injection of 3 c.c. of potassium chloride solution (2 Gm. per 100 c.c. per kilogram of body weight) established a hyperglycemia of such severity that it lasted for ninety minutes. This observation corresponds to the findings of Hochfeld[7] and others. The injection of 3 c.c. of a solution of calcium chloride (5 parts per 100 c.c. per kilogram of body weight) also provoked a hyperglycemia, but the latter was less accentuated than that caused by potassium chloride. Barath[8] and Schenk[9] report a hypoglycemia in the same type of experiment. Previous injection of atropine did not modify the hyperglycemia provoked by the injection of either potassium or calcium. Kiyohara[6] found that the hyperglycemia could not be induced by either potassium or calcium ions after the extirpation of both adrenals as it could before the extirpation. Kiyohara[6] attributed the hyperglycemia brought about by these ions to the action on the adrenals themselves. Hazard[10] studied the action of calcium and magnesium on potassium-provoked hypertension and hyperglycemia. He demonstrated that large doses of potassium chloride cause a discharge of adrenalin, which in turn produces a hypertension and a hyperglycemia. He showed that magnesium prevents potassium from exercising its adrenalin action while calcium allows it to provoke the adrenalin discharge but diminishes its hyperglycemic effect. The reduction of adrenalin glycosuria by calcium has been noted by various authors, some of whom even advocate the use of calcium salts in the treatment of diabetes in man. Among these, Asada[11] has shown that, in rabbits, repeated intravenous injections of small amounts of calcium chloride reduced the hyperglycemia due to adrenalin by 13 per cent. He also noted a 28 per cent smaller increase of blood sugar in rabbits which had been injected intravenously with 5 c.c. of 2 per cent potassium chloride solution, together with 3 mg. of adrenalin per kilogram of body weight subcutaneously, than with the same amount of potassium chloride. This question merits more study because the potassium in blood has been found to be higher in certain forms of diabetes. Further as to the effect of calcium on blood sugar, Cantarow, Brundage, and Housel[12] found that the intravenous injection of calcium gluconate and calcium levulinate in doses of 3.7 to 9.9 mg. of calcium ion per kilogram of body weight was followed by a decrease of 10 to 31 mg. per 100 c.c. in blood sugar within five to fifteen minutes. No inhibitory effect on insulin action was observed in the animal experiments of Fazekas and Himwich[13] when 10 mg. of calcium chloride or 10 mg. of magnesium sulfate were added to insulin prior to injection. It is interesting to note that although calcium, magnesium, and zinc belong in the same group in the Mendeléeff table, only zinc possesses the inhibitory effect on insulin action.

Nickel-Cobalt.—Bertrand and Macheboeuf[14] found the pancreas rich in nickel and cobalt. Labbe, Roubeau, and Nepveux[15] confirmed the results obtained by Bertrand and Macheboeuf[14] in their experiments on rabbits in which they showed that the addition to insulin of a trace of a nickel salt or of a cobalt salt intensifies and prolongs the hypoglycemic effect. Labbe, Roubeau, and Nep-

veux[15] then sought to determine the action of nickel and cobalt salts on the hypoglycemic power of insulin on diabetic individuals. They conducted two series of experiments. In the first series isolated injection of nickel chloride and cobalt chloride salts in isotonic solution into diabetic individuals with malnutrition and acidosis showed that the blood sugar level is not modified by cobalt and nickel salts. Identical injections made in normal subjects showed no modification in their glycemic levels.

In the second series of experiments they sought to find whether the injection of metallic solutions would influence the hypoglycemic power of insulin on diabetic individuals in the same manner as on animals. They administered half units of insulin per kilogram of body weight to eight diabetic individuals with acidosis and malnutrition, and to one diabetic patient without malnutrition. They varied the doses of nickel and cobalt chloride injected per kilogram of body weight, the first series of patients receiving 0.1 mg. of nickel chloride; the second series, 1 mg. of nickel chloride; the third series, 1 mg. of cobalt chloride. For each patient it was possible to make two series of observations, the first with insulin alone, the second with the same quantity of insulin added to the metallic solution. For each, the two curves of glycemia with and without nickel or cobalt salts were such that no reinforcement of the action of insulin, either in intensity or duration, could be noted. They found that the injection of cobalt or nickel salts without insulin, whether in healthy or in diabetic individuals, was without hypoglycemic effect as Bertrand and Macheboeuf[14] had shown to be the case with animals. They reported that action of insulin in association with nickel or cobalt salts on diabetic persons varied from that observed in healthy rabbits and dogs, a difference perhaps due to a dissimilar metabolism of the glucides in healthy animals and diabetic human beings. Magenta[1] in 1926 obtained in dogs results similar to those obtained by Blatherwick and Sahyun[16] in rabbits (1927). The latter reported a slight increase of glycemia when metallic salts alone were used. Metallic salts used simultaneously with insulin exercised little if any preventive action on the hypoglycemia of dogs, and never increased the hypoglycemic effect of insulin.

Zinc.—Hove, Elvehjem, and Hart[17] investigated the relationship of zinc to certain aspects of carbohydrate and protein metabolism in rats. Glucose tolerance tests were conducted by means of pipette-feeding of 0.2 Gm. of glucose per 100 Gm. of body weight after a five-hour starvation period. Tolerance curves obtained from zinc-deficient rats differed from rats on a normal diet. Liver glycogen determinations on both zinc-deficient rats and control rats revealed no difference in respect to liver glycogen. In the case of the zinc-deficient rats, these investigators found a distinct delay in the time interval necessary for the blood nitrogen to reach its maximum value. The distinct irregularity of the glucose tolerance curves obtained from zinc-deficient rats is explained as being due to a delay in the absorption rate of carbohydrate and protein from the gastrointestinal tract.

In dogs fed zinc oxide, D'Amore, Falcone, and Maramaldi[18] observed occurrences of severe glycosuria. Salant and Wise[19] also report glycosuria following the administration of zinc acetate and zinc malate, both orally and intravenously.

These observers explain this phenomenon on the basis of an increased permeability of the kidneys to sugar (renal diabetes).

Drinker, Thompson, and Marsh[20] state that glycosuria was not present in cats which had received zinc oxide in the form of a dry powder mixed with food. However, they did encounter glycosuria in two of three dogs subjected to the same method of zinc oxide administration. Curiously, gross and microscopic examination following autopsy revealed fibrous change in the pancreas of the zinc-treated cats and none in the pancreases of the zinc-treated dogs. No other organs of these animals showed pathologic change. The authors state that the amount of zinc oxide administered is greatly in excess of that usually encountered by man. Impressed by the fibrous changes in the pancreas of zinced cats, and knowing through their own researches the close chemical relationship between insulin and zinc, Scott and Fisher[21] estimated the insulin content and the zinc content of the pancreases of cats maintained on a basal diet, and also a basal diet to which zinc oxide had been added. They, too, found that the pancreas was greatly involved in the zinced cats through degenerative changes in the acinar tissues. Nodular pancreatic glands with bands of fibrous tissue replacing areas of acinar tissue were observed. The islet tissue of the pancreas in both the zinc and the control groups showed no change. Amyloid deposits in the spleen and calcification in the cortex of the adrenals were noted in many of the zinced cats. The liver and kidneys in both groups showed that a large amount of fat was present.

Since these changes occurred in both groups, it is not certain that zinc feeding was their specific cause. No changes were found in other tissues examined. They assert that heavy zinc feeding did not alter the total insulin content of the pancreas. Their results appear to indicate an increase in the amount of insulin per gram of pancreas, while the total insulin content remained unchanged. It was observed that on a basal diet the cat pancreas contains more zinc per gram of tissue than the liver.

The livers of zinc-fed cats contained nearly twice as much zinc per gram of tissue as the pancreases, although the cats on a basal diet contained a higher percentage of zinc per gram of tissue in the pancreas than in the liver. On the zinc diet insulin assays showed that 2.56 units of insulin were present per gram of pancreas, while on the basal diet only 1.68 units of insulin per gram of pancreas were present. The total insulin content of both groups was nearly the same (10.40 units on the zinc diet and 11.52 units on the basal diet).

Scott and Fisher[22] assayed the pancreases of nondiabetic human individuals who came to autopsy and found that 1.7 units of insulin per gram of pancreas were present. In diabetic individuals who came to autopsy, they noted that the pancreas contains less than 0.4 unit of insulin per gram of pancreatic tissue. It was observed that the average zinc content of the diabetic pancreas is one-half that of the normal pancreas. The zinc per gram of pancreas in the diabetic person assays at 0.07; in the nondiabetic person, the value is 0.14. No marked differences in zinc concentration in the livers of diabetic and nondiabetic individuals were observed. It is of interest to note the marked reduction of both insulin and zinc in the pancreas of the diabetic individual. Scott and Fisher[22]

suggest from this observation the possibility that a part of the zinc in the pancreas might be concerned with the storage and utilization of insulin.

Zinc Insulin Mixtures.—Zinc has been found to be present in the pancreas; it has also been found in all commercial preparations of insulin examined by Scott and Fisher.[23] Insulin solutions to which 0.02 per cent of zinc chloride are added, or equivalent amounts of zinc in the form of zinc acetate or zinc sulfate, demonstrated only 40 per cent of the original hypoglycemic activity when injected into mice. It was noted that convulsions occurred later in the group of mice that were given zinc insulin solutions than in the control group given insulin alone. This observation suggested that the action of insulin was delayed by the presence of the zinc salt. In rabbits, when the zinc insulin mixture was so adjusted that 0.24 per cent of zinc chloride was present, the action of insulin was also greatly delayed. These authors suggested that the zinc added to insulin altered the solution, so that a greatly delayed hypoglycemic action was produced. Rabbits receiving insulin alone yielded the lowest blood sugars two hours after the injection of insulin, and blood sugars reached the normal level in ten hours. Rabbits given zinc insulin showed a gradual fall in the blood sugar values during the first five hours, and blood sugar values were still below the normal level at the end of a ten-hour period. Injections of zinc sulfate solutions alone did not alter blood sugar response during an observation period of seven hours. It appears that the delayed hypoglycemic action of zinc-insulin mixtures is dependent upon some process which takes place in the solution before it is injected.

Fazekas and Himwich[13] observed no alteration of the typical action in either dogs or cats when insulin and zinc sulfate are injected independently into separate subcutaneous areas. Injections of zinc sulfate, the same concentrations as above but without insulin, had no effect whatsoever on blood sugar levels. When 0.9 per cent zinc was used in a zinc-insulin mixture, complete inhibition of insulin action resulted.

Copper.—Ussolzew[24] found that intravenous injections of 0.5 mg. and 1 mg. of copper nitrate caused a lowering of blood sugar in rabbits starved for twenty-four hours previous to injection. Tkachenko[25] found in rabbits that the administration of 0.1 mg. of copper per kilogram of body weight caused a hypoglycemia in rabbits.

Schnetz[26] observed the effect of copper on carbohydrate metabolism both through studies of the isolated liver and on the intact animal. His results show that copper causes a definite lowering of the ability of adrenalin to cause mobilization of glycogen from the liver, but does not disturb the normal glycolytic mechanism. He also found that the oral administration of 20 mg. of copper sulfate per day for a period of days in normal individuals reduced both the hyperglycemia caused by the injection of 0.5 mg. of adrenalin and the hyperglycemia produced by the administration of 30 Gm. of dextrose orally. In his experiments, equivalent amount of iron salts did not influence a hyperglycemia identically induced.

Copper salts were given over a period of weeks to four persons suffering from severe diabetes mellitus. A noticeable reduction in the amount of insulin necessary to control their diabetes was observed, as was also a lowering of blood

sugar and urinary sugar and a disappearance of acetone bodies. This action of copper in reducing blood-sugar is marked in cases of high blood sugar values. When blood sugar values are within normal limits, little effect is observed. These experiments (copper-adrenalin-dextrose) on normal individuals, and those made in the course of diabetes mellitus, appear to indicate that it is to hepatic action that copper-reduction of blood sugar values is due. This hypothesis is made on the basis of the following theory: copper administered by mouth is thought to be transported by the reticulo-endothelial system to the liver. Muller[27] believes that copper so alters the glycogenolytic mechanism of the liver that it does not respond in its usual way to adrenalin mobilization of glycogen action, with the resulting rise in blood sugar levels. Muller[27] believes that copper makes for stabilization of hepatic glycogen function. Schnetz[26] states as a hypothesis that copper is a catalyst which helps the oxidation of carbohydrates through hepatic or extrahepatic action.

Since at present we do not know where copper is distributed in the liver nor how long it remains in it, nor what chemical unions and reactions it engages in, we can only say that it appears that the real mechanism is not yet explained. The foregoing interesting theories must remain unproved hypotheses until more data have been made available.

Aluminum Insulin Mixtures.—When 11.2 mg. of aluminum chloride were mixed with ten units of insulin before injection, Fazekas and Himwich[13] observed no decrease in blood sugar values in the animals. When 5.6 mg. of aluminum chloride were mixed with ten units of insulin, a decrease in blood sugar values occurred, showing that the aluminum chloride at this concentration does not abolish insulin activity. Since Scott and Fisher[23] showed that potassium alum had no inhibitory effect on insulin, aluminum chloride apparently differs from potassium alum in its inhibitory effect on insulin.

Ferric Chloride Insulin Mixtures.—Maxwell and Bischoff[28] investigated the action of basic ferric salts in prolonging insulin hypoglycemia. Insulin was added to a dilute aqueous solution of ferric chloride which had been neutralized to the point of precipitation. In both rabbits and rats the addition of basic ferric chloride to insulin augmented the insulin action. The blood sugar of the control animals receiving insulin alone approached the normal at the fifth hour, while the blood sugar of the group that received insulin plus ferric chloride approached the normal value at the eighth hour.

Manganese.—Pignatari[29] noted the effect of manganese sulfate on the carbohydrate metabolism of rabbits. When 0.50 Gm. of manganese sulfate in physiologic solution per kilogram of body weight was injected daily, the animals died after the fourth or fifth injection. With doses of 0.10 Gm. of manganese sulfate, the animals remained alive for approximately one and one-half months. The first symptom of the manganese intoxication appeared after 0.80 Gm. of manganese sulfate had been administered. Pignatari[29] determined the fasting blood sugar level in each rabbit before the poisoning and on alternate days during the course of the intoxication. The method used for the determination of the blood sugar values was that of Bang-Condorelli.

The blood sugar values during the intoxication with manganese sulfate showed a decrease in the fasting blood sugar levels. In the final period of the

intoxication the glycemic level was so markedly decreased that in some cases the fasting blood sugar level was approximately one-half of the normal.

Pignatari[29] carried out glucose tolerance tests on these animals during the various stages of the intoxication with manganese sulfate. The blood sugar levels were determined until the fourth hour after the administration of the glucose. The principal characteristic of the glycemic reaction in rabbits intoxicated with manganese was the prolongation of the hyperglycemia. The hyperglycemia was due to the diminished capacity of the liver to absorb the circulating glucose and to synthesize glycogen. The hypoglycemia observed in the course of the intoxication with manganese can best be explained by a deficient and impaired glycolytic mechanism of the liver. Both the delayed hepatic glycogenesis and the deficient and impaired glycogenolysis can best be explained by changes produced by manganese sulfate on the liver.

Lead.—Von Ludwig Schmidt-Kehl[30] reports that rabbits fed a daily dose of 10 mg. of lead oxide per kilogram of body weight over a period of two or four months developed chronic lead poisoning. Five normal rabbits were used as controls. The administration of 2 per cent of the body weight of levulose in normal control animals, after a day of starvation, showed a rise of 16 per cent in the blood sugar values. In the five animals with chronic lead poisoning, the increase in blood sugar values rose to 35 per cent. These determinations were made according to the method of Hagedorn and Jensen. Histologic examinations revealed no pathologic changes present in either normal or leaded animals. It was concluded that in chronic lead poisoning of rabbits, even without the presence of any anatomic and histologic changes in the liver, there is already present undiscernible damage, as evidenced by increased blood sugar values in the leaded animals.

Cavagliano[31] observed the occurrence of glycosuria in patients during episodes of lead colic. He reports that the administration of 1.5 Gm. of glucose per kilogram of body weight to patients recovering from lead colic resulted in a glycosuria that manifested itself for a period of four hours. After considering alimentary glycosuria and renal glycosuria as possible explanations for this mechanism, he concludes, "The glycosuria indicates the functional disturbance of the liver caused by lead."

It has been demonstrated that injections of lead, whether in solution or in a colloidal state, localize chiefly in those organs that are rich in reticuloendothelial elements, and hence particularly in the liver. Colloidal lead, like mineral colloids in general, when injected, are prevalently deposited in the liver. The variations of the glycemic levels can be studied before and after the injection of these colloids.

Merlini[32] injected carefully standardized colloidal lead preparations into the marginal veins of rabbits. The normal glycemic levels of rabbits were determined in the control group. The blood sugar values varied from 116 mg. to a minimum of 81 mg. of glucose per 100 c.c. of blood. The average value of the blood sugar determinations in this control group was 83.9 mg. of glucose per 100 c.c. of blood. Merlini noted that rabbits which had received 0.021 Gm. of lead per kilogram of body weight after a few hours had blood sugar values

ranging from 54 to 58 mg. of glucose per 100 c.c. of blood. When the dose of the colloidal lead solution was reduced so that 0.01094 Gm. of lead per kilogram of body weight was injected daily for a period of five days, and then fasting blood sugar levels were determined, a hypoglycemia was noted. The level of this hypoglycemia was never found to be much below 50 mg. of glucose per 100 c.c. of blood. Merlini's data show that the rabbits reacted to colloidal lead with hypoglycemic reaction according to the dose administered.

Phosphotungstic Acid—Phosphomolybdic Acid.—Mukherjee[33] in 1925 noted the effects of the phosphotungstates and phosphomolybdates on carbohydrate metabolism when taken internally. Experimentally, phospho-24-tungstic acid, phosphomolybdic acid, molybdic acid, silicotungstic acid, sodium tungstate, and ammonium phospho-18-tungstate when taken by mouth lower the glycemic level in both rabbits and diabetic human beings. The experiment using diabetic patients was as follows: Three hours after administration of insulin there was an average blood sugar drop of 125.3 mg. per 100 c.c. of blood. Three hours after intake of 0.9 Gm. of phosphotungstic acid, an average blood sugar drop of 90 mg. per 100 c.c. of blood resulted. A diuretic action of these substances was noted while demonstrating the above hypoglycemic effects. An oxidizing action is observed when 0.1 Gm. of phosphomolybdic crystals in a 5 c.c. solution of benzidine in acetic acid turns blue, and becomes deeper in color when hydrogen peroxide is added. Benzidine and phosphomolybdic acid alone give a positive reaction. The allied substances give a positive reaction when added to alkaline phenolphthalein reagent. These substances, Mukherjee believes, act like oxidizing enzymes; and the oxidizing action associated with the hypoglycemic reaction suggests that oxidation of sugar in the tissues may produce hypoglycemia.

Mercury.—Richter[34] observed the presence of glycosuria associated with hyperglycemia in animals after the administration of sublimate of mercury. Frank[35] found that the intravenous injection of 3 mg. of bichloride of mercury into rabbits was followed by a marked glycosuria without change in the glycemic level.

Hunter and Roberts,[36] on the other hand, claim that in addition to the widespread tubular necrosis, distinct changes interpreted as evidences of injury can be demonstrated in the glomerular basement membranes of kidneys damaged by mercuric chloride. These alterations in the basement membrane may perhaps play an important role in the renal disturbances induced by the salts of mercury. In man, in the course of corrosive sublimate intoxication, Lepine[37] has observed glycosuria.

Uranium.—Lepine and Boulud,[38] Fleckseder,[39] Pollak,[40] Frank,[35] and Luzzatto,[41] reported a glycosuria variable in degree produced by uranium salts. In fasting animals, it was less and appeared later than in well-fed ones. In dogs, Frank[35] found that glycosuria prevailed from eight to eleven days after a single injection. Whitney[42] states that in the acute nephritis produced by uranium there is damage to the tubular epithelium of the kidneys and to the capillaries. There is generalized capillary damage resulting in decreased permeability of the walls. Reports as to sugar concentration in the blood varied from hyperglycemic to hypoglycemic levels.

Chromium.—Vernon,[43] first, and Kossa[44] later called attention to the production of glycosuria by chromium salts. Kossa's studies determined the amount of sugar in both the blood and the urine of animals subjected to his experiments. He found no hyperglycemia in animals poisoned with chromate. Other investigators report contradictory findings, both higher and lower than those of Kossa. Luzzatto[41] administered potassium chromate to well-fed and fasting animals and observed glycosuria and albuminuria following administration.

In addition to widespread tubular necrosis, distinct changes, interpreted as evidences of injury, can be demonstrated in the glomerular basement membranes of kidneys damaged by chromium.[36]

Tellurium.—Luzzatto[41] states that tellurium salts, in particular sodium tellurate, may cause glycosuria and albuminuria in animals, sometimes reaching 1 per cent sugar concentration. Albuminuria sets in first, followed within forty-eight hours after injection by a glycosuria which may last five days. Observations made on both fasting and well-fed animals indicated no increase in glycemic levels. Kaiwa[45] demonstrated that b-dimethyltelluronium dichloride, administered subcutaneously as well as intravenously, was effective in provoking hyperglycemia. The hyperglycemia first begins to appear one-half to one hour after injection, and lasts from two to four hours. Additional experiments were made on rabbits of which the splanchnic nerves had been bilaterally sectioned in order to observe whether the hyperglycemia due to b-dimethyltelluronium dichloride is of central origin. Results were identical, showing no involvement of the central mechanism. However, experiments on bilaterally suprarenalectomized rabbits showed a lower level of hyperglycemia than that found in either the normal or the splanchnicotomized animals.

COMMENTS

It is apparent that with the exception of sodium, potassium, nickel, and cobalt, the investigations of the direct effect of metals on carbohydrate metabolism have consisted, for the most part, of animal experimentation. Routine glucose tolerance tests in clinical cases of metal intoxications will probably yield interesting and significant information in this field. The data thus obtained will aid greatly in elucidating the physiologic and pathologic relationships of the metals and carbohydrate metabolism.

SUMMARY

1. The sodium ion depresses the blood sugar level in rabbits. In juvenile diabetic patients the administration of sodium salts decreases both blood sugar level and glycosuria. The mechanism of this action is unknown.

2. Potassium salts increased the blood sugar concentration and glycosuria of juvenile diabetic persons. This effect appears to be due to a stimulating effect on adrenalin discharge and is inhibited by magnesium.

3. There is no unanimity of opinion concerning the effect of calcium on the glycemic levels of various experimental animals. Calcium salts appear to depress the hyperglycemic effect of epinephrine in animals.

4. Nickel and cobalt salts, when added to insulin, delay the effect of the latter in normal rabbits and dogs, but not in diabetic human beings. Nickel and cobalt salts, when given alone, have no effect on blood sugar level of man or animals.

5. Zinc-deficient diet delays the absorption of carbohydrate from the gastrointestinal tract of rats. Zinc oxide has been found to produce glycosuria in dogs and pancreatic fibrosis in cats. The zinc content of the pancreases of diabetic persons is greatly below normal.

6. Zinc, aluminum, ferric chloride, nickel, or cobalt, when mixed with insulin, delays the action of the latter; calcium, magnesium, and potassium alum do not delay the effect of insulin when mixed with it.

7. Lead and manganese decrease the fasting blood sugar levels.

8. Phospho-24-tungstic acid, phosphomolybdic acid, molybdic acid, silicotungstic acid, sodium tungstate, and ammonium phospho-18-tungstate appear to cause a decrease in blood sugar of diabetic persons.

9. Glycosuria has been observed after the administration of mercury, uranium, and chromium.

10. The administration of copper salts, both in man and animals, causes a decrease in the ability of adrenalin to mobilize liver glycogen.

REFERENCES

1. Magenta, M. A.: Action du fluorure de sodium sur la glycemie, Compt. rend. Soc. de biol. **98:** 169, 1928.
2. Goldenberg: 3e Congres national de medecine, t.vi, fasc. 2, 663 Juillet, 1926.
3. Abelin, J., and Goldener, E.: Influence of Electrolytes on Action of Insulin, Klin. Wchnschr. **4:** 2446, 1925.
4. McQuarrie, I., Thompson, W. H., and Anderson, J. A.: Effects of Excessive Ingestion of Sodium and Potassium Salts on Carbohydrate Metabolism and Blood Pressure in Diabetic Children, J. Nutrition **11:** 77, 1936.
5. Rathery, F., and Bertoliatti, J.: Le potassium du sang dans les differentes formes du diabete sucre, Compt. rend. Soc. de biol. **117:** 875, 1934.
6. Kiyohara, K., Morita, M., and Muta, S.: De l'influence des ions K et Ca sur la teneur en sucre du sang, Compt. rend. Soc. de biol. **120:** 1011, 1935.
7. Hochfeld, O.: Ueber den Einfluss von Kalk- und Kalisalsen auf den Blutzucker, Ztschr. f. d. ges. exper. Med. **37:** 119, 1923.
8. Barath, E.: Calciumwirkung und Blutzucker, Klin. Wchnschr. **3:** 1618, 1924.
9. Schenk, P.: Ueber den Einfluss der intravenosen Infusion hypertonischer Natrium- und Calciumchloridlosungen auf den Blutzuckerspiegel, die Diurese und die Durchlassigkeit der Nieren, Ztschr. f. d. ges. exper. Med. **12:** 269, 1921.
10. Hazard, Rene: Action de l'ion calcium sur l'hypertension et sur l'hyperglycemie provoquees per l'ion potassium, Compt. rend. Soc. de biol. **121:** 1036, 1936.
11. Asada, T.: Einfluss des Kaliumchlorids auf die Adrenalinhyperglykamie und- lactacidamie, Mitt. a. d. med. Akad. zu Kioto **15:** 1140, 1935.
12. Cantarow, A., Brundage, J. T., and Housel, E. L.: Blood Sugar, Inorganic Phosphorus and Phosphatase Activity Following the Intravenous Injection of Calcium Salts, Am. J. Physiol. **115:** 1, 1936.
13. Fazekas, J. F., and Himwich, H. E.: Effect of Zinc and Aluminum on Hypoglycemic Action of Insulin, J. Pharmacol. & Exper. Therap. **58:** 260, 1936.
14. Bertrand, G., and Macheboeuf, M.: (1) Influence du nickel et du cobalt sur l'action exercee par l'insuline chez le chien, Compt. rend. Acad. d. sc. **183:** 5, 1926; (2) Nickel, cobalt et diabete, Compt. rend. Acad. d. sc. **183:** 257, 1926; (3) Sur la teneur relativement elevee du pancreas en nickel et en cobalt, Compt. rend. Acad. d. sc. **182:** 1305, 1926; (4) Influence du nickel et du cobalt sur l'action exercee par l'insulin, chez le lapin, Compt. rend. Acad. d. sc. **182:** 1504, 1926.
15. Labbe, M., Roubeau, H., and Nepveux, F.: Action de sels de nickel et de sur le pouvoir hypoglycemiant de l'insuline chez le diabetique, Compt. rend. Acad. d. sc. **186:** 181, 1928.
16. Blatherwick, N. R., and Sahyun, M.: Effect of Cobalt on Insulin Hypoglycemia in Rabbits, Am. J. Physiol. **81:** 560, 1927.
17. Hove, E., Elvehjem, C. A., and Hart, E. B.: Physiology of Zinc in Nutrition of Rat, Am. J. Physiol. **119:** 768, 1937.

18. D'Amore, L., Falcone, C., and Marmaldi, L.: Action toxique et alterations anatomiques produites par l'ingestion de l'oxyde de zinc, Compt. rend. Soc. de. biol. **44**: vol. 4: s. 9, 335, 1892.
19. Salant, W., and Wise, L. E.: The Production of Glycosuria by Zinc Salts, J. Biol. Chem. **34**: 447, 1918.
20. Drinker, K. R., Thompson, P. K., and Marsh, M.: Investigation of Effect of Long-Continued Ingestion of Zinc, in Form of Zinc Oxide, by Cats and Dogs, Together With Observations Upon Excretion and Storage of Zinc, Am. J. Physiol. **80**: 31, 1927.
21. Scott, D. A., and Fisher, A. M.: Studies on Pancreas and Liver of Normal and Zinc-Fed Cats, Am. J. Physiol. **121**: 253, 1938.
22. Scott, D. A., and Fisher, A. M.: Insulin and Zinc Content of Normal and Diabetic Pancreas, J. Clin. Investigation **17**: 725, 1938.
23. Scott, D. A., and Fisher, A. M.: Effect of Zinc Salts on Action of Insulin, J. Pharmacol. & Exper. Therap. **55**: 206, 1935.
24. Ussolzew, S.: Ueber den Einfluss von Kupferdarreichung auf den Blutzuckerspiegel, Biochem. Ztschr. **276**: 431, 1935.
25. Tkachenko, L.: Ueber den Einfluss von Metallsalze auf den Blutzuckergehalt, J. Orient. Med. **7**: 66, 1927.
26. Schnetz, H.: Ueber den Einfluss des Kupfers auf den Koklehydratstoffwechsel des Menschen, Ztschr. f. klin. Med. **129**: 739, 1936.
27. Muller, A. H.: Ausfuhrliche Literaturangaben über Kupfer, Med. Welt. **2**: 299, 1935; Ergebn. d. inn. Med. u. Kinderh. **48**: 444, 1935.
28. Maxwell, L. C., and Bischoff, F.: Augmentation of Physiologic Response to Insulin, Am. J. Physiol. **112**: 172, 1935.
29. Pignatari, F.: Glicemia e lipemia nella intossicazione de Manganese, Folia med. **18**: 484, 1932.
30. Schmidt-Kehl, L.: Ueber die Schädigung des Kohlehydratstoffwechsels der Leber bei chronischer Bleivergiftung, München. med. Wchnschr. **80**: 2003, 1933.
31. Cavagliano, B.: Glicosurie e diabete in rapporto alla medicina assicurativa, Med. del lavoro **20**: 407, 1929.
32. Merlini, E.: Ricerche sperimentali sulla glicemia nell'avvelenamento da piombo colloidale, Arch. Ist. biochim. ital. **5**: 395, 1933.
33. Mukherjee, H. N.: Hypoglycaemic and Other Actions of Phosphotungstic Acid. Phosphomolybdic Acid and Allied Substances, Biochem. J. **30**: 1583, 1936.
34. Richter, P. F.: Zur Frage des "Nierendiabetes," Deutsche. med. Wchnschr. **25**: 840, 1899.
35. Frank, E.: Ueber experimentelle und klinische Glykosurian renalen Ursprungs, Arch. f. exper. Path. u. Pharmakol. **72**: 387, 1913.
36. Hunter, W. C., and Roberts, J. M.: Glomerular Changes in Kidneys of Rabbits and Monkeys Induced by Uranium Nitrate, Mercuric Chloride, and Potassium Bichromate, Am. J. Path. **8**: 665, 1932.
37. Lepine, R.: Les glycosuries toxiques, Arch. de méd. expér. et d'anat. path. **15**: 129, 1903.
38. Lepine, R., and Boulud: Sur L'absence d'hyperglycemie dans la Glycosurie uranique, Rev. med. **24**: 1, 1904.
39. Fleckseder, R.: Ueber Hydrops und Glykosurie bei Uranvergiftung, Arch. f. exper. Path. u. Pharmakol. **56**: 54-67, 1906-07.
40. Pollak, L.: Kritisches und Experimentelles zur Klassifikation der Glykosurieen, Arch. f. exper. Path. u. Pharmakol. **61**: 376, 1909.
41. Luzzatto, R.: Die Glykosurie bei experimentellen Nephritiden, Ztschr. f. exper. Path. u. Therap. **16**: 18, 1914.
42. Whitney, C.: Capillary Permeability in Acute Uranium Nephritis, J. Path. & Bact. **31**: 699, 1928.
43. Vernon: These de Paris, 1885, cited by J. R. Lepine, Le diabete sucre, Paris, 1909, F. Alcan.
44. Kossa, J.: Ueber Chromsaure-Diabetes, Arch. f. d. ges. Physiol. **88**: 627, 1902.
45. Kaiwa, T.: Hyperglycaemic Action of B-dimethyltelluronium Dichloride, Tohoku, J. Exper. Med. **20**: 163, 1932.

ALLERGY IN RELATION TO PURPURA*

J. Warrick Thomas, M.D., and J. R. Forsythe, M.D., Cleveland, Ohio

SIR WILLIAM OSLER[1] in 1914 reported a series of purpura cases compiled over a period of nineteen years. He stressed the possible relationship of purpura with erythema multiforme or the erythema group, and pointed out the association of skin lesions and visceral symptoms. Alexander and Eyermann[2] in 1927 reported two patients in whom purpura was definitely related to the eating of various foods, as these patients were free from symptoms on the elimination of the incriminated food. In 1921 Duke[3] reported a patient with severe abdominal pain associated with purpura and angioneurotic edema in whom there was a strong maternal and paternal familial history of purpura. Sensitization studies revealed marked reactions to a number of foods, and the purpura as well as the angioneurotic edema and abdominal complaints cleared up following a program of dietary restrictions. Peshkin and Miller,[4] in their review of the literature, reported that purpura has been known to follow the administration of such drugs as mercury, aniline, iodine, pyridine, quinine, belladonna, ergot, acetphenetidin, salicylic acid, and arsphenamine.

Eyermann[5] observed 18 cases in which purpura followed ingestion of certain foods. He stated that regardless whether the purpura and associated symptoms appear to be induced by infection, by ingestion, or by inhalation, the common factor appears to be a disorder of the capillary mechanism which leads to congestion, exudation, or hemorrhage. It may also lead to skin lesions, such as erythema multiforme, urticaria, angioneurotic edema, and purpura. In addition, he feels that exudations into the stomach or intestinal walls cause nausea, vomiting, and abdominal colic.

There are a number of other authors including Squier and Madison,[6-8] Rowe,[9] and Brown,[10] who have contributed materially to the subject of purpura as related to allergy.

DISCUSSION OF ALLERGIC PURPURA

In a series of 64 patients with purpura hemorrhagica observed in the Cleveland Clinic, ten were thought to have a possible allergic factor related to their problem, and for this reason a complete allergy survey was done. The patients were analyzed from the viewpoint of the various purpuric manifestations, as well as for the correlation of the age incidence and sex.

There were four males and six females in the series. It was observed that the age factor did not play a prominent part, as there was an equal distribu-

*From the Department of Allergy, Cleveland Clinic.
Presented at the Mid-West Forum on Allergy at Chicago, January 14, 1940.
Received for publication, March 20, 1940.

TABLE I

DISTRIBUTION OF VARIOUS ALLERGIC MANIFESTATIONS ASSOCIATED WITH PURPURA HEMORRHAGICA AS RELATED TO FAMILY HISTORY, BLOOD FINDINGS,* AND OTHER ASSOCIATED CLINICAL MANIFESTATIONS

CASE NO.	ALLERGIC MANIFESTATIONS	FAMILY HISTORY	TOURNIQUET TEST	R.B.C. MILLIONS	HB. %	W.B.C. THOUSANDS	COAGULATION TIME—MIN.	BLEEDING TIME MIN.	CLOT RETRACTION MIN.	PLATELETS
1	Urticaria Gastrointestinal allergy	Positive	Not done	4.21	65	12.5	2	4	Normal	560,000
2	Urticaria Rhinitis	Positive	Positive	4.78	81	4.25	5	2½	Normal	100,000
3	Urticaria Rhinitis	Positive	Not done	4.87	75	6.25	10	3	Normal	310,000
4	Urticaria Gastrointestinal allergy Rhinitis	Positive	Not done	5.29	81	4.20				Normal
5	Urticaria Gastrointestinal allergy Rhinitis	Positive	Positive	4.20	84	6.20				
6a	Rhinitis (1)	Negative	Positive	5.00	81	10.70	6	7½	Normal	510,000
	(2)			5.15	78	10.75	5	4½	Normal	270,000
7b	Rhinitis	Negative	Positive	3.80	78	7.40	10	2	Normal	300,000
8	Atopic dermatitis Gastrointestinal allergy	Positive	Not done	4.35	75	9.70		15†	Not done	40,000
9c	Asthma	Positive	Negative	4.70	71	13.5	12	2	Normal	Normal
10	Asthma Hay fever Urticaria	Positive	Positive	4.68	71	5.65	10	20†	None	20,000

*Normal averages as follows:
Coagulation time = 5 to 10 minutes.
Clot retraction = 1 to 2 hours.
Platelet count = 250,000 to 500,000.
Bleeding time = Up to 3 minutes.
a. Chronic nephritis; intestinal hemorrhage.
b. Chronic dental sepsis; acute nephritis; chronic prostatitis.
c. Essential hypertension; multiple myeloma; scabies; ptosis of kidney.

tion of patients in the first, second, third, fourth, and seventh decades. Seven of the patients had tourniquet tests performed, and of this group five gave characteristic petechiae.

From the point of view of the different purpuric manifestations, 9 had petechiae, 7 had ecchymotic spots, 4 had recurrent attacks of epistaxis, 3 had bleeding gums, 2 had bloody stools, 2 had an abnormal catamenia, and one had had a cerebral hemorrhage.

PURPURA AND RELATED ALLERGIC MANIFESTATIONS

Table I shows the distribution of the various allergic manifestations associated with purpura hemorrhagica as related to family history, blood findings, and other associated clinical manifestations. There were only two patients who had a definite thrombopenia in addition to their allergy (see Cases 8 and 10). A detailed review of the ten cases follows:

CASE 1.—A boy, 2 years old, first visited the clinic in 1936. His mother stated that three months prior to admission, some abdominal rumbling with mucus and blood in the stools was noted. This cleared up after a week's duration, and was followed two days later by edema of the arms and legs and by purplish spots in the skin. Since that time he has had recurrent attacks of purpura with associated swelling of the legs. There had been considerable nasal congestion in addition to frequent colds. A bilateral family history of allergy was elicited. Sensitization studies were made, and the child was found to be allergic to a number of the more common foods and inhalants. His therapeutic program consisted of the avoidance of contact with inhalants and a program of dietary restriction. Three months later he was reported greatly improved without having had subsequent purpuric lesions and with a marked improvement of his nasal symptoms.

CASE 2.—A woman complained of recurrent hives for a period of five years. During the thirteen months prior to her admission, urticarial lesions had been present almost constantly. She had experienced recurrent exacerbations of the urticarial lesions following the ingestion of orange juice. Sensitization studies were made, and a program of avoidance or elimination of the reacting inhalants, foods, and molds controlled the urticarial lesions. Two years later, following a tonsillectomy, she had some bleeding from the rectum, nose, and gums, and an increased tendency to bleed easily. Tourniquet test was positive. Sensitization studies were repeated, but she did not materially improve on this program until after taking thyroid extract and Lugol's solution for a number of years.

CASE 3.—A woman, aged 32 years, was suffering from abdominal cramps, diarrhea, and mucus in the stools. She stated that certain foods, such as tomatoes, peaches, and cantaloupes, aggravated these symptoms. Further history revealed a perennial allergic rhinitis and a history of eczema. She had allergy studies at this time and was placed on a program of dietary restrictions and advised to avoid inhalants. Two weeks after returning home she developed purpuric symptoms about the right knee; these were thought to be due to some allergic factor that was not entirely controlled. The symptoms later cleared up satisfactorily.

CASE 4.—A woman, 65 years old, gave a history of recurrent purpuric spots for a period of twelve years prior to her examination. The lesions occurred mostly on the legs and thighs. There was a history of asthma which cleared up on a program of hyposensitization. She was of the opinion that nervous strain, sugars, pastries, and such foods as strawberries and tomatoes caused the purpuric spots to appear. She gave positive reactions to food, inhalant, and bacteria testing, and was placed on a restricted diet and instructed to avoid inhalants to which she reacted. When last seen, fourteen months after the institution of treatment, she reported that she had been getting along well and had had no recurrence of her purpura.

CASE 5.—A woman, aged 37 years, complained of a recurrent purpuric rash over her abdomen for the past several years. Eight weeks prior to admission she had observed this rash appearing over her arms and about her ankles. No known etiologic factors were elicited. She had had some stinging irritating lesions, presumably hives, following contact with hollyhocks, and on one occasion when changing feathers from one pillow to another. She gave a history of perennial rhinitis and frequent colds, especially during the winter months. She frequently experienced heartburn after eating white bread and pancakes. There was a definite family history of allergy. Physical examination revealed nothing of clinical significance other than the purpuric areas over her arms, thighs, and legs. Tourniquet test was positive and special blood examinations were within normal limits. She was placed on a restricted diet and told to avoid inhalants to which she reacted.

CASE 6.—A 16-year-old boy was first seen on March 3, 1939. He reported that in September, 1938, he worked in a new store for two days, a total of twenty-eight hours, during which time tar was being heated in the basement to repair the roof. The tar fumes were very strong during the entire period he was working. Within four or five days following exposure to the tar fumes, he developed severe cramps in his legs. This was followed by nausea and vomiting, and by black stools without diarrhea. Two weeks after his exposure he first developed reddish-purple spots of varying size on his arms, legs, and lower abdomen, and considerable edema of the feet. Within the next eight weeks (spent entirely in bed) the purpura was noted in successive crops. Hematuria was then discovered. On December 12, 1938, severe edema of the face became apparent, with the onset of more petechiae. On March 1, 1939, he again developed nausea and vomiting, with bloody diarrhea and hematuria, and the onset of a new crop of petechiae.

The boy had been having a chronic catarrh for many years which was worse during the winter months. He has noted definitely that being in contact with dogs and cats tended to make his nasal condition worse, and that the catarrh improved when he stayed away from them. Since September, 1938, hives have been noted at infrequent intervals. Eating of cooked cabbage, oatmeal, and several other cooked vegetables definitely caused nausea and vomiting. There was conclusive family history of allergy. The boy's paternal grandmother had chronic eczema, a paternal uncle has hay fever, and one paternal cousin has asthma. *By further questioning, the fact was elicited that as a child the patient had chewed tar in large wads upon numerous occasions.* Routine sensitization studies were performed which revealed very strong reactions to a number of the more common inhalants, molds and bacteria. Very strong reactions were likewise noted to a number of the more common foods and sea foods. Patch test to mild tar ointment in a lanolin base gave a slight erythema (patch test to lanolin alone was negative), while a patch test to crude tar ointment gave a very strongly positive reaction. The patient was placed on an allergy program consisting of dietary restriction, avoidance and hyposensitization with the positive inhalants to which he reacted. He was further advised to stop smoking entirely and to keep a food diary.

He was seen at weekly intervals for a period of six months and continued his program of hyposensitization during that period.

The urine examination of the patient revealed repeated specimens showing albumin varying from one to four plus, both granular and hyalin casts in varying numbers, and red and white blood cells. The entire picture showed a definite improvement, and the last several specimens showed only a trace of albumin, three to five red and white blood cells, and rare granular cast on microscopic examination.

A definite improvement in symptoms characterized by a feeling of general well-being, increased appetite, and a weight increase of 15 pounds after the first month, was observed. He continued for several weeks to have scattered purpuric lesions over the lower extremities, which gradually disappeared. He was last seen nine months after the treatment was instituted, and at that time his allergy was very well controlled. He had had no recurrence of his purpuric lesions. Urine examination, however, revealed few red blood cells. He has gained 50 pounds in weight since treatment was first started. We feel that he has a chronic nephritis.

CASE 7.—A man in his 60's gave a history of having had recurrent episodes of epistaxis, mainly during the winter months. Purpuric or ecchymotic spots were noted over the legs for a month prior to his visit to the clinic. He gave a history of recurring attacks of nasal obstruction, postnasal drip, and rhinorrhea for thirty years. The purpuric lesions were noted over the lower extremities and abdomen. He gave minimal reactions to the various allergens with the exception of bacteria, to which he gave very marked delayed reactions—more so than is usually encountered.

The patient's urine was examined at daily intervals during his period of hospitalization, which was of ten days' duration. Significant findings were albumin, varying from 1 to 3 plus. Both hyalin and granular casts were seen on microscopic examination with both red and white blood cells from zero to eight in number. There is no question of an acute nephritis in this case.

He was placed on a program of dietary restrictions and hyposensitization. He did not follow his program and we did not hear from him since outlining his treatment until recently, when his doctor reported his death due to chronic nephritis.

CASE 8.—A little girl, 2½ years old, had an atopic dermatitis showing the characteristic distribution. Dietary restriction cleared up the eczema satisfactorily without recurrence, but not the purpura. Since there had been a definite persistence of the purpuric lesions along with a thrombopenia, a splenectomy was performed. The patient is doing well with no recurrence of purpura over a three-month period, her platelet count being 90,000. Her purpura in retrospect was not related to allergy. The atopic dermatitis responded satisfactorily to treatment.

CASE 9.—A man, 30 years old, gave a history of recurrent crops of ecchymotic areas over his lower extremities for a period of five months. In addition, he stated that he had had asthma practically all his life. His only suggestion relative to the purpura was that he had been taking some white homeopathic pills which he had started about the time of the onset of symptoms. He further stated that he had had periods in which he was symptom-free, during the time that he was receiving the medicine. There was a very definite familial history of allergy. Physical examination revealed purpuric lesions over both lower extremities, with an associated scabies infection involving the trunk and groin.

There was some frank edema of the arms and legs, and his blood pressure was systolic 164 and diastolic 116 and later dropped to systolic 160 and diastolic 104. His urine showed a faint trace of albumin only on two occasions. Roentgenograms showed singular dense nodular areas in both lower lungs, and no definite etiology of this process could be determined. His eleventh and twelfth ribs revealed numerous tumor areas, and later he had pathologic fractures of these ribs. This was felt to be a possible multiple myeloma. Pyelograms were made, and the right kidney was found ptosed just below the rim of the pelvis. He received x-ray treatment over the ribs, and following this he showed some general improvement. Continued treatment over a period of months showed a definite improvement with a subsidence of the edema. It was very questionable as to whether his purpura was related to his allergy; however, the purpura cleared after several months.

Sensitization studies were done, and he was found allergic to a number of the common foods, inhalants, and molds. He was placed in a strict dietary regime, including the elimination of drugs, and instructed regarding the avoidance of inhalants. He was seen eighteen months after the original program of treatment was outlined and reported that he had been free of purpuric symptoms, and that he had observed a recurrence of his asthma only following the ingestion of milk.

CASE 10.—The other patient with a thrombopenia was a woman in her 20's who had definite seasonal (ragweed) hay fever and asthma, as well as urticaria and perennial rhinitis. She was placed on a program of pollen and inhalant hyposensitization. Her history revealed purpuric lesions for twelve years. She had an exacerbation of her purpuric symptoms following strenuous exercise, and on one occasion had a transient hemiplegia for several days. She did not follow recommendations regarding therapy, and her allergy symptoms have continued. Purpuric symptoms persisted with a low platelet count, and a splenectomy was advised. The splenectomy was performed and since then there has been no recurrence of purpura.

COMMENTS

The question of allergy has to be ruled out as an etiologic factor in purpura hemorrhagica. When a patient presents a frank allergy at the time of the purpuric lesions, such an allergy should be investigated and controlled if possible. When patients fail to respond to general therapeutic measures, even though there is no history of allergy, an allergy investigation is warranted, as well as in the case presenting only a family history of allergy.

Our study brings out very conclusively that an inhalant factor should be considered as a possible cause in some cases, and that when such a factor has been controlled the patient may respond to treatment as shown in Case 6.

It has been definitely proved that a program of dietary restrictions alone will control certain cases (Case 4).

SUMMARY AND CONCLUSIONS

1. Allergic manifestations, a personal history of allergy, or a family history of allergy warrants an allergy investigation as a possible etiologic factor in purpura hemorrhagica.

2. When all other methods of treatment have proved unsuccessful, an allergy investigation is warranted as a possible etiologic factor.

3. Of 64 patients with purpura hemorrhagica ten had complete allergy surveys as a possible allergic etiologic factor. Of these ten a positive family history of allergy was elicited from eight.

4. Thrombopenia was observed in two of the ten patients presenting a definite allergic problem.

5. One or more allergic manifestations were found to be present in each of the ten patients studied.

6. Petechiae were observed in nine patients and ecchymotic areas were observed in seven. These were the most frequent purpuric lesions demonstrated.

7. Two patients presented frank and common allergic manifestations but failed to show any improvement in their purpuric symptoms until after splenectomy. In both of these cases the platelet count was definitely reduced.

8. Chronic nephritis was present in two patients studied.

REFERENCES

1. Osler, Sir William: The Visceral Lesions of Purpura and Allied Conditions, Brit. M. J. **1**: 517, 1914.
2. Alexander, H. L., and Eyermann, C. H.: Food Allergy in Henoch's Purpura, Arch. Dermat. & Syph. **16**: 322, 1927.
3. Duke, W. W.: Food Allergy as a Cause of Abdominal Pain, Arch. Int. Med. **28**: 151, 1921.
4. Peshkin, M. M., and Miller, J. A.: Quinine and Ergot Allergy and Thrombocytopenic Purpura, J. A. M. A. **102**: 1737, 1934.
5. Eyermann, C. H.: Allergic Purpura, South. M. J. **28**: 341, 1935.
6. Squier, T. L., and Madison, F. W.: Thrombocytopenic Purpura Due to Food Allergy, J. Allergy **8**: 143, 1937.
7. Madison, F. W., and Squier, T. L.: Etiologic Factors in Hemorrhagic States, Wisconsin M. J. **37**: 636, 1938.
8. Madison, F. W., and Squier, T. L.: Leukothrombopenia With Increased Capillary Permeability, J. A. M. A. **112**: 879, 1939.
9. Rowe, A. H.: Clinical Allergy Due to Foods, Inhalants, Contactants, Fungi, Bacteria and Other Causes: Manifestations, Diagnosis and Treatment, Philadelphia, 1937, Lea & Febiger, pp. 469-473.
10. Brown, O. H.: Purpura Hemorrhagica From Food Sensitization; Successful Treatment by Dietary Regulation and Use of Digestants; Case Report, Southwestern Med. J. **19**: 131, 1935.

EFFECT OF SULFAPYRIDINE ON STAPHYLOCOCCUS TOXIN*

An in Vivo Study in the Rabbit

R. H. Rigdon, M.D., Anne Haynes, B.S., and Alys Lipscomb, A.B.
Memphis, Tenn.

CARPENTER and Barbour[1] observed that staphylococcus toxin was inactivated in vitro by sulfanilamide. They found that 92 per cent of 630 mice survived when injected intra-abdominally with a sulfanilamide-staphylococcus toxin mixture. All 195 controls died when given toxin alone. These investigators also noted that the in vivo action of staphylococcus toxin was less marked when sulfanilamide was given either before or subsequent to the toxin. Carpenter, Barbour, and Hawley[2] likewise found that 89 per cent of 400 mice injected intraperitoneally with a sulfanilamide-staphylococcus toxin mixture survived while all of a group of 100 controls that received only the toxin died.

Rigdon and Freeman[3] observed that sulfapyridine had no effect on the hemolytic, the skin necrotizing, and the lethal factors in staphylococcus toxin. They did note, however, a bacteriostatic action produced by this drug on staphylococci in vitro and also a variation in the degree of the action of sulfapyridine on three different strains of staphylococci.

The effect of sulfapyridine on the lethal action of staphylococcus toxin is studied in the present experiment.

MATERIALS AND METHODS

Fifteen cubic centimeters of a 25 per cent solution of sulfapyridine† in a 50 per cent dextrose solution were injected into the marginal ear veins of 28 normal adult rabbits. A similar quantity of 50 per cent dextrose was given to 18 animals. Five-tenths gram of sodium sulfapyridine in 5.0 c.c. of saline was injected intravenously into each of five rabbits. Thirty minutes following the injection of the sulfapyridine each rabbit was given either 0.15 c.c. or 0.3 c.c. of staphylococcus toxin intravenously. For one of the control observations 34 rabbits were given a corresponding amount of staphylococcus toxin. For a second control 10 rabbits were given 2,800 units of staphylococcus antitoxin intravenously and thirty minutes later 0.3 c.c. of staphylococcus toxin. The time of death was recorded. Animals surviving for forty-eight hours or longer were considered protected against the lethal effects of this toxin.

*From the Department of Pathology and Chemistry Laboratory, Division of Medicine, University of Tennessee, Memphis.

Aided by grants from the Council on Pharmacy, American Medical Association; John and Mary R. Markle Foundation and a special grant from the University of Tennessee.

Received for publication, March 22, 1940.

†The sulfapyridine in dextrose and the staphylococcus toxin and antitoxin were supplied by Lederle Laboratories. Merck & Co. supplied the sodium sulfapyridine.

The amount of free sulfapyridine in the blood was determined by the method of Marshall and Litchfield.[4] Blood was removed from the heart at the following intervals: thirty minutes, and one, two, three, five, and eight hours. Groups of four rabbits were bled at alternate intervals. The average sulfapyridine determination was used to establish the points on the curve (Chart 1).

TABLE I

EFFECT OF SULFAPYRIDINE ON LETHAL ACTION OF STAPHYLOCOCCUS TOXIN

(All injections were made intravenously.)

NUMBER OF RABBITS	SULFAPYRIDINE	AMOUNT OF STAPHYLOCOCCUS TOXIN (C.C.)	AVERAGE WT. (GM.)	NO. AND TIME* OF DEATH FOLLOWING INJECTION OF TOXIN	
24		0.3	2,030	Group A	19
				Group B	2
				Group C	1
				Survived	2
20	15 c.c. of 25% sulfapyridine in 50% dextrose	0.3	2,578	Group A	14
				Group B	5
				Group C	0
				Survived	1
13	15 c.c. of 50% dextrose	0.3	2,493	Group A	3
				Group B	4
				Group C	1
				Survived	5
10		0.15	1,334	Group A	3
				Group B	3
				Group C	2
				Survived	2
8	15 c.c. of 25% sulfapyridine in 50% dextrose	0.15	1,315	Group A	0
				Group B	3
				Group C	4
				Survived	1
5	15 c.c. of 50% dextrose	0.15	1,558	Group A	1
				Group B	2
				Group C	1
				Survived	1
5	0.5 Gm. of sodium sulfapyridine in 5 c.c. of saline	0.15	1,390	Group A	1
				Group B	4
				Group C	0
				Survived	0
10	2,800 units of staphylococcus antitoxin	0.3	1,870	Group A	0
				Group B	0
				Group C	0
				Survived	10

*Group A—Rabbits dying from 0-2 hours following injection of the toxin.
Group B—Rabbits dying from 2-12 hours following injection of the toxin.
Group C—Rabbits dying from 12-48 hours following injection of the toxin.

EXPERIMENTAL

The number of rabbits used in each group, the amount of sulfapyridine in dextrose, dextrose alone, sodium sulfapyridine in saline, staphylococcus antitoxin, and staphylococcus toxin, and the number of rabbits that died at the different intervals are shown in Table I. It is evident from these data that neither sulfapyridine in dextrose nor sodium sulfapyridine in saline prevents death produced by an intravenous injection of staphylococcus toxin. A total of 2,800 units of staphylococcus antitoxin, given thirty minutes before the lethal dose of toxin, completely protects rabbits against the toxin.

Chart 1 shows the concentration of free sulfapyridine in the blood after an injection of sulfapyridine in dextrose. The rate of excretion appears to be very rapid. The free sulfapyridine in the blood at specific intervals after an intravenous injection of 0.5 Gm. of sodium sulfapyridine in saline is as follows: thirty minutes, 13.2 mg. per cent; one hour, 13.3 mg.; two hours, 9.2 mg.; three hours, 4.46 mg.; and five hours, 2.4 mg.

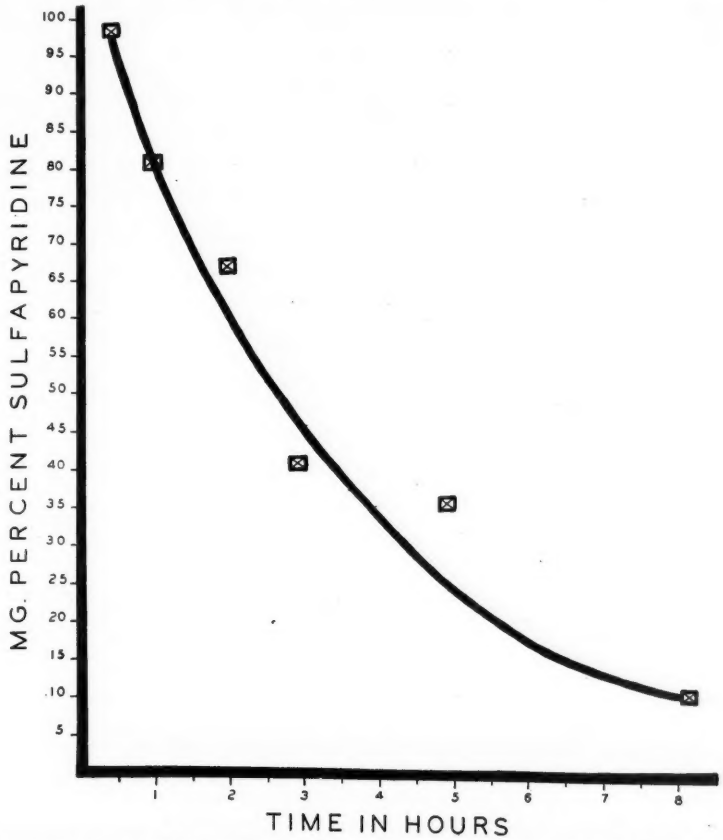


Chart 1.—Amount of free sulfapyridine in the blood of rabbits following an intravenous injection of 15 c.c. of a 25 per cent solution of sulfapyridine in 50 per cent dextrose.

It is interesting to observe in TableI that rabbits given 15 c.c. of a 50 per cent solution of dextrose and 0.3 c.c. of staphylococcus toxin thirty minutes later, live for a longer time than those animals given either the sulfapyridine in a similar quantity of dextrose and 0.3 c.c. of staphylococcus toxin or the toxin only. The rabbits given both the sulfapyridine in dextrose and only the dextrose bled very freely from the venous puncture following each injection. There was no difference in either the bleeding or the clotting time of these rabbits when compared with the controls.

Two rabbits died while they were being injected with the sulfapyridine in dextrose. One died while receiving the dextrose. The ear in which both the sulfapyridine in dextrose and the dextrose were injected became edematous after several minutes. Extensive necrosis of the ears usually occurred in those rabbits that survived the lethal action of the toxin.

DISCUSSION

The data presented here show that sulfapyridine has no inhibitory effect on the lethal action of staphylococcus toxin when given intravenously to the rabbit. Taylor and his associates[8] are of the opinion that when sulfapyridine is given in dextrose to man the drug circulates in the blood in a form different from other sulfapyridine preparations. If such occurs in the rabbit, one would then conclude from these experiments that it also has no effect on the lethal action of staphylococcus toxin.

There is a significant difference in the twelve-hour survival rate of the rabbits given dextrose and 0.3 c.c. of staphylococcus toxin over those injected with only the toxin. This difference is two and one-half times its standard error. A larger number of rabbits given dextrose and 0.3 c.c. of staphylococcus toxin lived more than two hours longer than those animals injected with sulfapyridine in dextrose and a similar quantity of toxin. There is no difference in the low hour survival rate, however, in the rabbits given sulfapyridine in dextrose and toxin over those animals given only the staphylococcus toxin.

The failure of the rabbits that received the sulfapyridine in dextrose to survive for a longer time than the rabbits given only the toxin suggests that the effect of the sulfapyridine, plus that of the toxin, is sufficient to produce death more quickly than the toxin alone. A phenomenon similar to this was observed by one of us (R. H. R.) in mice given sulfapyridine and staphylococcus toxin. Eighteen mice in a group of 30 died when a mixture of sulfapyridine and toxin was given intraperitoneally, while only 15 died in a group of 30 when given only the staphylococcus toxin.

The results of this experiment suggest that the hypertonic dextrose solutions may inhibit the union of staphylococcus toxin with tissue cells. One of us (R. H. R.) recently studied hemolysis produced by staphylococcus toxin on rabbit red blood cells suspended in hypertonic solution of sodium chloride and found an inhibition in the rate and the degrees of lysis which paralleled the concentration of the salt.[5] In another study it was observed that necrosis failed to occur at the site of intradermal injection of staphylococcus toxin when a 5 per cent solution of sodium chloride was previously injected into the local area of the rabbits' skin. Necrosis occurred in these rabbits below the point of injection of the toxin. It was thought that "the inhibitory action of sodium chloride on the dermal necrosis produced by staphylococcus may be the result of the action of the salt on the tissue cells. The change effected may inhibit the union of the toxin with the cells. This union apparently is responsible for the necrosis."[6]

Smith[7] has observed that sucrose, glucose, glycerol, and ethylene glycol inhibit the necrotic action of staphylococcus toxin. This inhibition appears to be related to the hydroxyl content rather than to the molecular weight. The mechanism of the action is one of partial destruction of the toxin. The staphylococcus toxin in our experiment must not have been destroyed, since the total number of deaths within a period of forty-eight hours in the group of rabbits receiving the dextrose and the toxin and the group receiving only the toxin show no significant difference.

It appears that the rate of excretion of sulfapyridine in dextrose following an intravenous injection in the rabbit is similar to that which occurs in man.[8]

The results of this experiment suggest that sulfapyridine would have no effect on the toxin liberated by certain strains of staphylococci in vivo. The pathologic lesions caused by toxin-producing strains of staphylococci in vivo are similar to those lesions produced by staphylococcus toxin which was prepared in vitro and then injected locally into experimental animal.[9, 10] Any effect that sulfapyridine might have on a staphylococcus infection apparently would be other than by its action on the toxin.

CONCLUSIONS

Sulfapyridine does not inhibit the lethal action of staphylococcus toxin in the rabbit.

Rabbits given an intravenous injection of 50 per cent dextrose and thirty minutes later staphylococcus toxin survive for a longer time than those animals given only staphylococcus toxin.

REFERENCES

1. Carpenter, C. M., and Barbour, G. M.: Inactivation of Toxins of Staphylococcus aureus and Clostridium welchii in Vitro by sulfanilamide, Proc. Soc. Exper. Biol. & Med. **41**: 354, 1939.
2. Carpenter, C. M., Barbour, G. M., and Hawley, P. S.: The Inactivation of Staphylococcal Toxin in Vitro by Sulfanilamide, J. Pediat. **14**: 116, 1939.
3. Rigdon, R. H., and Freeman, P.: The Effect of Sulfapyridine on Staphylococci and Staphylococcus Toxin, J. LAB. & CLIN. MED. **25**: 1125, 1940.
4. Marshall, E. K., and Litchfield, J. T.: The Determination of Sulfanilamide, Science **88**: 85, 1938.
5. Rigdon, R. H.: Inhibitory Effect of Hypertonic Solutions of Sodium Chloride on Staphylococcus Hematoxin, J. Infect. Dis. **60**: 25, 1937.
6. Rigdon, R. H.: Inhibitory Action of Sodium Chloride on Skin Necrosis Produced by Staphylococcus Toxin, Arch. Path. **22**: 763, 1936.
7. Smith, M. L.: The Influence of Glycerol on the Skin Necrosis Produced by Staphylococcus Toxin, Brit. J. Exper. Path. **18**: 265, 1937.
8. Taylor, F. H. Laskey, Lowell, Francis C., Adams, Margaret A., Spring, William C., and Finland, Maxwell: A Comparative Study of the Blood Concentrations and Urinary Excretion of Sulfapyridine and Sulfanilamide After Single Doses of Sulfapyridine and Related Compounds Administered by Various Routes, J. Clin. Investigation **19**: 201, 1940.
9. Rigdon, R. H.: Local Lesions Produced in Mice by Staphylococcus Toxin and by Toxin and Non-Toxin Producing Strains, Surgery **6**: 91, 1939.
10. Rigdon, R. H.: Hemolysis Produced by Staphylococcus Colonies and Toxin on Agar Media Containing Various Animal Bloods, J. LAB. & CLIN. MED. **24**: 1264, 1939.

ELECTROCARDIOGRAPHIC CHANGES FOLLOWING THE INTRAVENOUS ADMINISTRATION OF MAGNESIUM SULFATE*

III. Combined Effect With Digitalis

J. Roscoe Miller, M.S., M.D., and T. R. Van Dellen, M.S., M.D.
Chicago, Ill.

IT HAS been previously shown by means of the electrocardiogram that magnesium when given intravenously has a definite effect on the cardiac conductive system.[1] This effect consists of an early acceleration followed by slowing, with delay in the auriculoventricular and intraventricular conduction time and increased excursion of all complexes. It has been shown that this action of magnesium upon the conductive system is not central but direct.[2]

Zwillinger[3, 4] showed that magnesium had a beneficial but temporary effect on digitalis intoxication. He found it useful in combating ectopic impulses from any source, paroxysmal tachycardia, ventricular fibrillation and heart block. He believed that magnesium had no untoward effect on an already damaged conduction system. He was able to save rabbits who had been given lethal doses of digitalis with repeated small doses of magnesium. All control animals died. Similar studies were done on frogs. Block and Pick[5] confirmed the clinical studies of Zwillinger, citing a single case. Smith, Winkler, and Hoff[6] concluded from their studies on magnesium that Zwillinger's work has little physiologic support since such arrhythmias develop spontaneously or can be evoked in the presence of a greatly increased concentration of magnesium in the serum.

The present problem was undertaken to ascertain the effect of magnesium sulfate, as measured by the electrocardiogram, on the conductive system of the digitalized dog.

METHOD

As in previous experiments[1, 2] the unanesthetized dog was placed on the left side and electrodes were attached to the right front and left rear legs, thus corresponding to the conventional second lead. Electrocardiograms were made on a string galvanometer type machine. Both magnesium sulfate and digitalis were injected into the right saphenous vein.

EXPERIMENTS

Each dog underwent three separate experiments at no less than weekly intervals. These consisted of (1) the administration of magnesium sulfate, (2) digitalis, and (3) digitalis followed in twenty minutes by magnesium.

*From the Department of Medicine, Northwestern University Medical School, Chicago.
Received for publication, March 29, 1940.

Experiment 1.—Magnesium was administered intravenously, and the results obtained were in agreement with those previously reported.

Dog 1, weight 13.6 kg., received 10 c.c. of a 20 per cent magnesium sulfate solution given at the rate of 2 c.c. per minute (Fig. 1). The rate was at first accelerated following by slowing. The P-R interval increased from 0.16 to 0.18 second, and the QRS interval from 0.04 to 0.06 second. The T-waves originally inverted became diphasic.

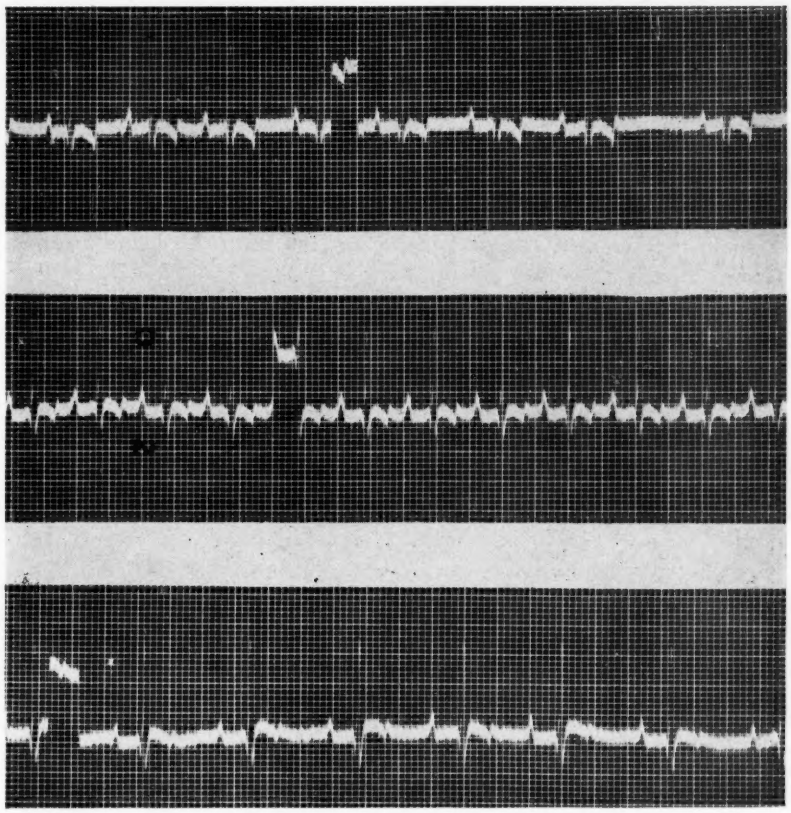


Fig. 1.—Dog 1. *A*, Normal graph. *B*, Following the injection of magnesium sulfate. Note increase in rate (130). *C*, Twenty minutes after injection.

Dog 2, weight 20.5 kg., received the same amount of magnesium sulfate. The changes in rate were not unlike those of Dog 1. The P-R interval increased from 0.14 to 0.18 second, and the QRS interval from 0.05 to 0.07 second.

Experiment 2.—In this experiment digitalis (Digalen) was given intravenously as rapidly as possible. It was found that approximately 1⅓ cat units per kilogram of body weight were sufficient to produce definite electrocardiographic changes. These changes consisted of bradycardia, slowing of the auriculoventricular and ventricular conduction times, and increased excursion of the T-wave. The only objective evidence of toxicity was vomiting, although as much as 28 cat units were given intravenously.

Dog 1 received 20 cat units of digitalis (Digalen). The rate fell from 80 to 60 beats per minute (Fig. 2). The P-R and QRS intervals increased from

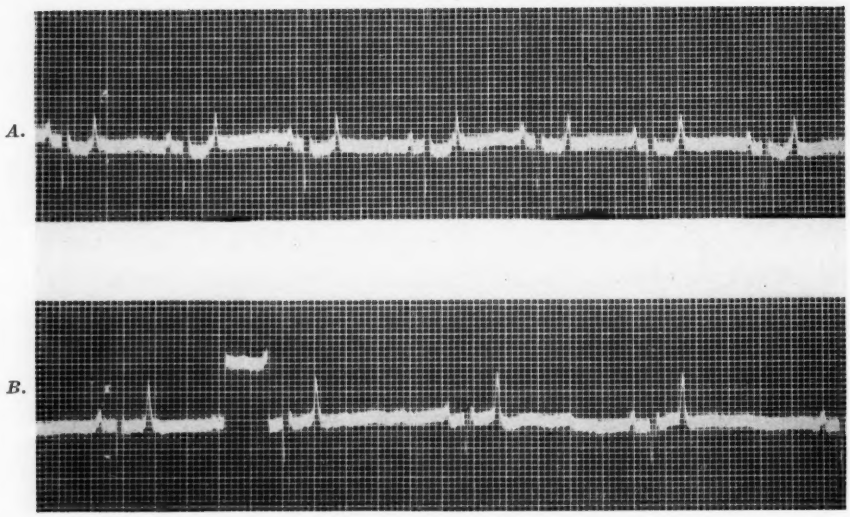


Fig. 2.—Dog 1. *A*, Normal graph. *B*, Twenty minutes after the intravenous administration of 20 cat units of digitalis (Digalen). Note the bradycardia, increased P-R interval, and elevation of the T-wave.

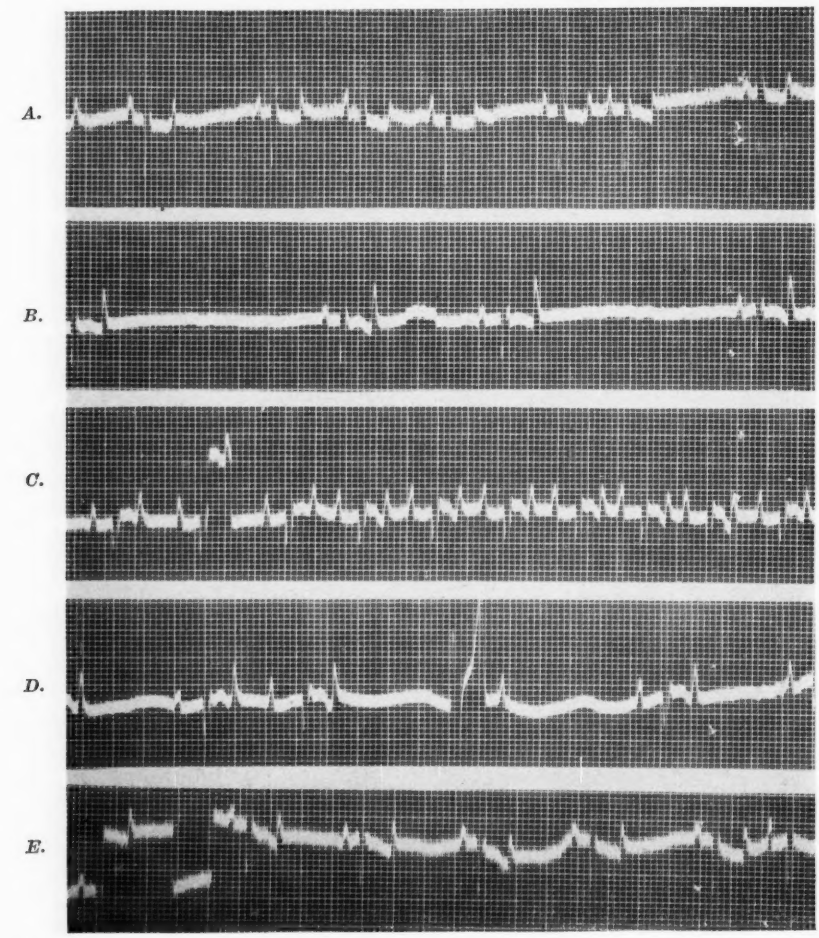


Fig. 3.—Dog 1. *A*, Normal graph. *B*, Twenty minutes after injection of 20 cat units digitalis (Digalen). *C*, Following injection of magnesium sulfate solution. *D*, Twenty minutes later. P-R interval now 0.20 second. *E*, Eighteen hours from beginning of experiment.

0.11 to 0.13 second and 0.04 to 0.05 second, respectively. The T-wave became more upright by 100 per cent. These changes were still present at the end of twenty-five minutes, at which time the experiment was terminated.

Dog 2 received 28 cat units of digitalis. The P-R interval increased from 0.16 to 0.18 second, but the QRS complexes were unaffected. The T-wave became more sharply inverted. A marked sinus arrhythmia occurred.

Experiment 3.—The same amount of digitalis was then given to each animal as in previous experiments, followed in twenty minutes by the same amount of magnesium sulfate. Following the administration of digitalis, tracings similar to those in Experiment 2 were obtained.

After the administration of magnesium the rate in Dog 1 increased from 40 to 100 beats per minute, following which it became slower, but at no time did it approach the bradycardia attained with magnesium only (Fig. 3). The P-R interval, which had been 0.12 second at the beginning of the experiment, increased to 0.14 second following administration of digitalis, and to 0.20 second following magnesium. The latter auriculoventricular conduction time is far in excess to that obtained in Experiments 1 and 2. A marked sinus arrhythmia which had developed following the administration of digitalis almost disappeared following the administration of magnesium; however, numerous premature ventricular contractions were now present. An electrocardiogram made eighteen hours later was not unlike that seen at the beginning of the experiment, except for a slight prolongation of the P-R interval most likely of digitalis origin.

When magnesium was administered to Dog 2, the P-R interval, which was originally 0.16 second and had been elevated to 0.18 by digitalis, was increased to 0.28 second. Instead of the sinus arrhythmia which followed the administration of digitalis, a 2:1 heart block developed.

In Dog 3 magnesium not only failed to control a 2:1 heart block produced by digitalis, but also prolonged the auriculoventricular conduction time from 0.16 to 0.22 second, and the ventricular conduction time from 0.06 to 0.07 second. In Dog 4 digitalis produced an occasional 2:1 heart block, but following the administration of magnesium a continuous block developed. The T-waves became sharply inverted, and the auriculoventricular conduction time increased from 0.20 to 0.22 second.

These results do not agree with the clinical reports of Zwillinger. Magnesium sulfate given to the digitalized dog failed to regulate the sinus arrhythmia and actually provoked the occurrence of premature beats and heart block. Magnesium sulfate is also capable of prolonging the auricular conduction time in excess of that resulting from digitalis or magnesium alone. An initial increase in rate was noted, followed by slowing, but the rate was more rapid than that produced by digitalis. This apparently demonstrates that magnesium overcomes the vagal stimulation of digitalis.

CONCLUSIONS

1. In the digitalized dog magnesium sulfate is capable of altering the contour of the T-waves and producing a further delay in auriculoventricular conduction time in excess of that of digitalis or magnesium alone.

2. In the digitalized dog magnesium sulfate causes a brief increase in rate, followed by a slowing which approaches that obtained by digitalis. It is thus capable of overcoming the vagal stimulation produced by digitalis but for short periods only.

3. In the digitalized dog magnesium sulfate is capable of producing impulses of ectopic origin.

4. The results indicate that magnesium sulfate does not overcome the effects of digitalis intoxication but increases the degree of block and the occurrence of ectopic impulses.

REFERENCES

1. Miller, J. Roscoe, and Van Dellen, T. R.: Electrocardiographic Changes Following the Intravenous Administration of Magnesium Sulfate. An Experimental Study on Dogs, J. LAB. & CLIN. MED. **23**: 914, 1938.
2. Van Dellen, T. R., and Miller, J. R.: Electrocardiographic Changes Following the Intravenous Administration of Magnesium Sulfate. II. An Experimental Study on Dogs. J. LAB. & CLIN. MED. **24**: 840, 1939.
3. Zwillinger, L.: The Effect of Magnesium on the Heart, Klin. Wchnschr. **14**: 1429, 1935.
4. Zwillinger, L.: The Effect of Magnesium Sulfate on Cardiac Intoxication Due to Strophanthin (Strophanthus Preparation), Wien. klin. Wchnschr. **49**: 594, 1936.
5. Block, C., and Pick, A.: The Effect of Magnesium on Automatic Ventricular Rhythm in Digitalis Intoxication. Clinical Study of Heterotopic Formation of Stimulus, Wien. Arch. f. inn Med. **29**: 435, 1936.
6. Smith, P. K., Winkler, A. W., and Hoff, H. E.: Electrocardiographic Changes and Concentration of Magnesium in Serum Following Intravenous Injection of Magnesium Salts, Am. J. Physiol. **126**: 720, 1939.

COMBINATIONS OF LEAD, ARSENIC, AND OTHER CHEMICALS WITH EXPERIMENTAL ASPHYXIA OF TUMORS

FREDERICK M. ALLEN, M.D., NEW YORK, N. Y.

OTHER papers[1] have described the effects of prolonged local asphyxia of normal parts and also of tumors, especially the transplantable carcinomas and sarcomas of rats and mice. The most striking effects on tumors are (a) dark hemorrhagic congestion of the tumor in contrast to the bright arterial hyperemia of the normal tissues of the limb after ligation, and (b) extensive necrosis of the tumors, sometimes to the point of complete cure, without permanent damage to the normal tissues. Subsequent papers[1a] will describe similar powerful effects of temporary asphyxia on spontaneous tumors, including the complete cure of one patient with cancer of the skin. While the ligation method is necessarily unsuited for any extensive use in practical tumor treatment, there is experimental evidence[2] of its ability to augment the effect of x-ray treatment. Other attempted combinations have been less promising, but even the negative results appear worth recording for the sake of theoretical information on the tumor problem.

Chemotherapy might be advanced by the finding of a substance which would show at least a partial selectivity for tumor tissue, and which by acting as an adjuvant to asphyxia would make the destruction of tumors safer and more

Received for publication, April 1, 1940.

efficient. According to the theory of Neuberg and Caspari,[3] the hyperemia following ligation should strengthen the chemotherapeutic action by carrying systemically injected substances in larger proportion to the tumor area and in smaller proportion to other parts of the body. For locally injected drugs there is the advantage of lying for several hours in direct contact with the tumor, with diffusion perhaps aided by altered permeability and asphyxial injury of the tumor cells.

ARSENIC

Arsenic in topical applications has been used to cure some tumors since the time of the Egyptians[4]; it is also capable of producing tumors.[5, 6] Its pharmacology is of particular interest in the present connection. Cushny[7] states that "arsenic is a capillary poison, dilating capillaries and permitting edema formation more readily than the normal. Arsenites and arsenious acid do not coagulate proteins or change them in any way. . . . It seems to act only upon living cells, and unlike acids and alkalies, forms no combination with the dead tissues."

Sollmann[8] says, "Arsenical compounds act locally as mild and slow corrosives, and have been employed in superficial cancer. Systemically, they relax the capillaries and increase their permeability, thus stimulating inflammation." Arsenic is said to be toxic to all animals with a central nervous system, and to some lower forms, but it cannot be classed as a general protoplasmic poison. It has little effect on ferments, although "it is now generally believed that the arsenicals hinder protoplasmic oxidation in an unknown way." Arsenic is described as causing peripheral vasodilatation and paralysis of capillaries. "This view is favored by the fact that they have become more permeable. Intravenous injections of large quantities of salt solution will cause edema in animals poisoned with arsenic, but not in the normal. Since increased permeability of the capillaries is one of the essential features of inflammation, the phenomena of arsenic poisoning are similar to those produced by an irritating inflammation, although the primal cause is different. Arsenic produces nephritis, at first mainly vascular, but always with more or less inflammation of the epithelium." These statements, are supported by references to the well-known works of Magnus, MacNider, and others.

Sollmann further states: "Arsenicals are not precipitant and therefore irritate only weakly, even on wounds and mucous membrances, but the cells die slowly after prolonged contact. They are sometimes used for killing dental nerves, and as salves for superficial epitheliomas. Arsenic is much more destructive to pathologic cells, so that the cancerous tissue may be killed without injury to the surrounding normal tissue. As with all cancer salves, some of the deeper tumor cells usually escape, leading to inoperable recurrences."

Because the effects of arsenic so closely resemble those of asphyxia, arsenic was selected for a long series of systemic and constitutional treatments of rat tumors. Beginning with the pharmacopeial Fowler's solution, containing 1 per cent arsenic trioxide, decimal dilutions were made with physiologic saline to contain from 1 mg. to 0.00001 mg. per cubic centimeter.

Systemic Treatment.—Willberg's[9] scale of arsenic tolerance of different species omits the rat. Experience has indicated that the lethal dose is near 1 mg.

per 100 Gm. of weight, with slight variations according to age, obesity, etc., but much more according to general strength. As weak animals have a decidedly reduced arsenic tolerance, animals with large tumors or with ligation treatment demand smaller doses than the normal. Those in best condition could receive as high as 0.9 mg. per 100 Gm., but the usual doses were smaller. Acute death from arsenic has nothing to do with organic lesions in the liver, kidneys, or elsewhere. In harmony with the above-mentioned pharmacologic action of arsenic, the picture resembles shock, and acute arsenic poisoning is perhaps correctly classified under shock. At least in the rat, the elimination is evidently rapid and the danger of cumulative effects is slight. Thus, even tumor-bearing rats were able to receive doses of 0.6 or 0.7 mg. twice or sometimes three times in twenty-four hours and to continue this sometimes more than two weeks without serious symptoms. Feeding the drug was not attempted. Subcutaneous injections produce slow ulcerations. Therefore intraperitoneal injections were usually employed.

The tumor experiments may be summarized as follows: Positive results were obtainable with single arsenic injections followed by single ligations, inasmuch as sloughing of all visible tumor tissue followed ligations of only two or three hours, which is never possible with ligation alone. Similar effects were obtained with various combinations of repeated arsenic injections and repeated ligations. A few cures were positive, in the sense of indefinite survival without tumor recurrence. But with all kinds of combinations having any decisive effect upon the tumors, the mortality was overwhelmingly high, as has been the usual rule with all chemotherapeutic attempts in the literature. Inasmuch as a lower mortality and a higher proportion of cures were obtainable with ligation alone, the combination with systemic arsenic administration did not appear promising.

Similar experiments with single and repeated arsenic injections were performed with the chicken sarcoma. Willberg[9] gives the tolerance dose of arsenic for chickens as 0.06 Gm. per kilogram. The single doses in tumor-bearing chickens ranged only from 5 to 7 mg., but these were sometimes continued for as long as nine days. Results were positive only to the extent that sloughing of tumors after a given duration of ligation was more extensive with the arsenic injections than without. The general results were inferior to those in rats, and no cures were obtained.

Local Arsenic Injections.—Although arsenic produces remarkably little irritation in the normal tissues, it may, as already mentioned, give rise to ulcers when injected subcutaneously. As should naturally be expected, the necrotizing effect is increased by asphyxia. An approximate scale of tolerance was worked out in normal rats for combinations of one to five hours of asphyxia with arsenic dilutions from 10 mg. to 0.0001 mg. per cubic centimeter; for example, the undiluted Fowler's solution (10 mg. per cubic centimeter) caused ulceration with one hour of ligation and fatal gangrene with two hours. The concentration of 1 mg. per cubic centimeter was compatible with ligation to the limit of three hours, but caused gangrene with four hours. The solution of 0.1 mg. per cubic centimeter and weaker dilutions could be tolerated with ligations of four and

five hours. A modifying factor was later encountered, in that the quantity or location of the injection may create an increased pressure in the tissues, which, if continued, increases the tendency to gangrene.

The results with tumors may be summarized as follows: Without ligation, rat tumors are not destroyed by single local arsenic injections in any of the concentrations mentioned, or by fractional quantities infiltrated every fifteen minutes as as to keep the tumor thoroughly bathed in the solution for three or four hours, even if epinephrine or ephedrine is added. With any length of ligation, an arsenic dilution of 0.00001 mg. per cubic centimeter resembles plain saline solution in showing no perceptible effect, but all stronger solutions had an influence in destroying tumors. Cures of sarcoma 39 were obtained with ligations of two to three hours preceded by injections ranging from 0.2 c.c. of 10 mg. arsenic per cubic centimeter to 5 c.c. of 0.0001 mg. per cubic centimeter solution. In successful cases the arsenic not only shortened the time of ligation but also sharpened the specificity; the tumors were killed with less damage to the normal tissues than from ligation alone, and the resulting ulcers healed much more rapidly. Statistics are meaningless because success appeared to be governed entirely by one mechanical condition. The rat tumors are firmer in consistency than the surrounding normal tissues, so that a satisfactory infiltration of the tumor is difficult to obtain and the fluid tends to accumulate particularly in the loose subcutaneous tissue. If pressure is used to compel penetration, the normal tissues become subject to gangrene. If the first treatment is only partially successful, a repetition is practically impossible, because the injection fluid cannot be retained in an open ulcerating tumor, though collodion coating and other devices were used in the attempt. For this reason, the permanent cures, though individually rather spectacular, were comparatively few among the sarcomas and were absent with the Flexner-Jobling carcinoma and the Walker tumor.

For the same mechanical reason, attempts to combine ligation and arsenic injection in the treatment of spleen, kidney, and other intra-abdominal tumors all failed. On the other hand, a small series of scrotal and testicular sarcomas turned out favorably. The testis offers a special condition because of its firm tunics, and the strong reaction of the scrotum to irritation (as mentioned previously under ligation alone)[1] perhaps conduced to the cures which were obtained, with ligations of thirty to ninety minutes preceded by injection of 0.5 to 1 c.c. solution of 0.01 to 0.1 mg. arsenic per cubic centimeter. In all these cases, however, the testes became atrophied and the animals were sterile in breeding tests. Therefore, a cure with selective preservation of the testis was not obtained.

A small series of mice, with sarcoma 180 of 0.8 cm. diameter in the legs, were treated by injection of the tumors with 0.5 c.c. solution containing 0.001 mg. arsenic per cubic centimeter, followed by ligation for twenty, forty, sixty, eighty, and one hundred minutes, respectively. The tumors ligated for twenty and forty minutes merely ulcerated; the others were permanently cured. A single trial of forty minutes' ligation preceded by infiltration with 0.5 c.c. of a stronger arsenic solution (0.01 mg. per cubic centimeter) resulted in a cure.

Arsenic injections combined with ligation of the chicken sarcoma contributed to the breakdown of the tumor tissue but in no case led to a cure.

LEAD

The phenomena described by Bell[10] and others, of swelling, hyperemia, and more or less necrosis in tumors under lead treatment are suggestively similar to the results of ligation. Although it is the consensus of later work that clinical cures are not thus obtained, the use of local asphyxia to reinforce the systemic lead administration appeared attractive. Thanks are due to Dr. William H. Kraemer for supplying his preparations of colloidal lead phosphate on two occasions, the first time with a small admixture of manganese, and the second time plain.

A series of 39 rats, with medium or large tumors, were not able to tolerate as high dosage as described by Kraemer.[11] The stronger animals, of 150 to 200 Gm. weight, often died from intravenous injections above 1.5 c.c. of the suspension, containing 4 mg. of metallic lead per cubic centimeter. For all animals it was found safest to divide doses of 1 c.c. or more into two equal injections, morning and evening. Even with these precautions, administration at the rate of about 2 mg. of lead per 100 Gm. weight daily was never tolerated for more than two or three days, and rats commonly died within a few days after the last injection. The explanation may lie in the relation between the general strength and resistance to lead. The tumor rats may have had some impairment of vigor even when they appeared in good condition, and when they were perceptibly weak or anemic their tolerance of lead was much lower.

The tumors were not perceptibly affected by small doses of lead within the limits of safety. With dangerously large or fatal doses the sarcoma and carcinoma (but not the Walker tumor) sometimes showed softening and more or less necrosis, but there was no instance of cure with survival.

When the tumor-bearing legs were ligated for periods of one to three hours in connection with the lead treatment, there were indications of an increase of necrosis in the tumors without increase of damage to the normal leg tissues. In cases where the rats survived, this necrosis appeared so complete as to give promise of cure. The purpose had been to achieve a cumulative effect of the lead and ligation upon the tumors. The dominant result was actually a cumulative effect upon the animals, the ligated ones dying from doses which could be tolerated by nonligated controls. No cures were obtained.

A few attempts were also made to combine the lead treatment with intraperitoneal or intravenous arsenic injections, given during a different time in the same day, in the hope that a nontoxic dose of arsenic might have an adjuvant action with a nontoxic dose of lead to destroy a tumor. The same sort of failure resulted, because one poison lowered the tolerance of the animal for the other.

Remarks in the literature, attributing the deaths of lead-treated rats to anemia, are erroneous. Numerous additional experiments were performed, in which single or repeated transfusions were given before or after the lead treatment, or the lead suspension was mixed with fresh heparinized rat blood for injection. It is a simple matter by these means to overcome anemia entirely, even in animals which were weak or anemic before the treatment, but there is no particular alteration in the mortality.

It is fair to note that the action of a given substance is not always the same toward different tumors and in different species. Therefore, if the claims of partial tumor changes can be verified with nonfatal doses of lead in clinical cases, there may still be a possibility of its use as an adjuvant to other methods of treatment.

OTHER COLLOIDS

On account of the statements of authors, such as Neuberg and Caspari,[3] concerning the tumor-killing action of various metals, and also the emphasis placed by other writers upon the special advantages of the colloid state, much time and many animals were devoted to an investigation of intravenously injected substances other than lead. Particular attention was paid to colloidal arsenic, partly because of the reports of Hendrick and Burton.[12] The other colloidal solutions included gold,[13] silver, iron,[14, 15] manganese, copper, antimony, sulfur, and selenium.[16] Attempts were made by combinations and alternations of these to obtain a cumulative action upon the tumors, at the same time avoiding the toxicity of the individual elements, but failure resulted because the general toxic action was cumulative. In particular, it was hoped that an advantageous combination with ligation might be found by injecting immediately before ligating so that the circulating material would be held in the tumor for several hours, or by injecting immediately after release of the tourniquet so that the reactive hyperemia would flood the tumor with a maximum amount of the material. In either instance it was hoped that the asphyxiated tumor might be sensitized to the poison. All results were negative, without a single cure. Such cures as might have been expected from ligation alone were prevented by the fact that the intoxicated animals could not survive the usual duration of ligation.

DYES

Dyes have been among the most favored chemotherapeutic agents in attempts to influence cell respiration or anchor toxic substances to the tumor.[14] It has been customary to search for semidiffusible substances, which would pass from the blood into the tumor and remain there for a sufficient period of time. The special conditions of ligation, already mentioned, place a premium upon the freest possible diffusibility and provide favorable opportunities for any selective action.

No experiments were performed with systemically injected dyes. A few trials were made with single or repeated local injections of dilute aqueous solutions of eosin, methylene blue,[17] or gentian violet, during ligation of different rat tumors, mouse sarcoma 180, and chicken sarcoma. No cures and no improvement in results were obtained. Irregularities in distribution of the dyes were plainly visible. Addition of eosin or methylene blue to the usual arsenic solutions gave no improvement of results as compared with arsenic alone. These dyes may have been poorly chosen for diffusibility or other qualities, and the results may perhaps be improved under better technical conditions.

OTHER AGENTS AFFECTING RESPIRATION

Attempts to attack the tumor cell through its respiration have been too numerous to review.[14, 18] The earliest stage of this research in the Physiatric Institute included keeping the animals in glass chambers under various reduc-

tions or increases of oxygen pressure. Later some trials were made with chemical agents, such as potassium cyanide, to depress respiration, and with dinitrophenol to increase cell metabolism.[19] Oxygen enrichment was also undertaken by subcutaneous and intraperitoneal injections of oxygen several times in each of the twenty-four hours, which sufficed to maintain an abnormally bright color of the venous blood. Chronic carbon monoxide poisoning was established by this same injection method, using ordinary illuminating gas. Preliminary tests determined the minimum lethal doses of these injections for normal animals, which could thus be kept saturated to such a point that the blood remained cherry red and death resulted from a slight increase of the injections. The only new principle involved was the systemic injection of the above agents in connection with local ligation, as well as local injections of the solutions and gases directly into the region of the ligated tumor. Although these experiments in the aggregate represented much work, the results were so completely negative as to require no detailed description.

STREPTOCOCCUS TOXIN

A few intravenous injections of Coley fluid[20] were tried, together with a larger number of local infiltrations with this fluid, or living or killed streptococcus cultures. Special attention was given to repeated infiltrations, and particularly to various combinations with ligation. The tissue reaction in the rat is different from that in man, so that the indurated swellings which are familiar clinically[1a] are not obtainable in the rat. Neither a curative tendency nor the slightest indication of a selective toxicity of the streptococcus products for tumor as compared with normal tissues was found.

Streptococcus toxin can best be classified along with arsenic in its action upon the blood vessels. The principal error in dealing with such remedies has consisted in the search for a specific toxic action on tumor cells. The correct approach must place emphasis upon the vascular and tissue reactions, which vary with animal species, tumors, and locations of the tumor. Negative results in rats, therefore, by no means exclude the usefulness of either arsenic or streptococcic toxin under properly chosen clinical conditions, as will be shown in a subsequent publication.[1a]

CONCLUSIONS

1. The action of certain drugs, especially arsenic, and to a less extent lead, strikingly resembles that of local asphyxia and the mechanism of the tumor-killing effect may be similar. The attempts to use local ligations to obtain an intensified action of the drugs, or to use the drugs in local or systemic application to augment the effect of asphyxia, on the whole gave scanty success because of insufficient specificity, the distinctly increased injury to the tumors being offset by increased local or systemic dangers.

2. In conformity with previous work indicating that the tumor-killing action of local ligation is due neither to the direct asphyxia nor to the excess oxygenation of the ensuing hyperemia, the combination of ligation with various agents serving to increase or to depress cell respiration proved negative.

3. Notwithstanding the generally inconclusive results, some emphasis should be placed on the fact that cures with arsenic were obtained in some mouse

tumors and also in favorably situated rat tumors. There are undoubtedly differences in efficacy, depending upon the location and the specific sensitiveness of different tumors, and an application of this experience for an improved arsenic therapy of certain clinical tumors will be described elsewhere.[1a]

REFERENCES

1. Allen, F. M.: Local Asphyxia and Temperature Changes in Relation to Gangrene and Other Surgical Problems, Tr. A. Am. Physicians **52:** 189, 1937.
   Ligation and Refrigeration of Intestine, Surgery **3:** 893, 1938.
   The Tourniquet and Local Asphyxia, Am. J. Surg. **41:** 192, 1938.
   Resistance of Peripheral Tissues to Asphyxia at Various Temperatures, Surg. Gynec. & Obst. **67:** 746, 1938.
   Effects of Ligations on Nerves of the Extremities, Ann. Surg. **108:** 1088, 138.
   Physical and Toxic Factors in Shock, Arch. Surg. **38:** 155, 1939.
   Surgical Considerations of Temperature in Ligated Limbs, Am. J. Surg. **45:** 459, 1939.
   Experiments Concerning Ligation and Refrigeration in Relation to Local Intoxication and Infection, Surg. Gynec. & Obst. **68:** 1047, 1939.
   Mechanical Treatment of Venomous Bites and Wounds, South. M. J. **31:** 1248, 1938.
   Observations on Local Measures in the Treatment of Snake Bite, Am. J. Trop. Med. **19:** 393, 1939.
   Transfusions and Polycythemia in Normal and Tumor-bearing Rats, J. LAB. & CLIN. MED. **25:** 471, 1940.
   Influences of Local Asphyxia, Organ Extracts and Temperature Changes on Engrafted Subcutaneous and Visceral Tumors in Rats, Urol. & Cutan. Rev. **44:** 1, 1940.
   Local Asphyxia and Tumors, Am. J. Surg. **49:** 484, 1940.
   Ligation Treatment of Chicken Sarcoma, Med. Rec. **152:** 87, 1940.
   Action of Ergot in Normal and Tumor-Bearing Rats, Med. Rec. **152:** 351, 1940.
   Possible Factors in the Reaction of Tumors to Local Asphyxia, Am. J. Clin. Path. **10:** 813, 1940.

1a. Allen, F. M.: Effects of Local Reaction in Spontaneous Tumors of Animals and Man, Arch. Surg. **41:** 79, 1940.

2. Allen, F. M.: Local Asphyxia and Roentgen Irradiation of Transplantable Rat Tumors, Am. J. Roentgenol. **42:** 745, 1939.

3. Neuberg, C., Caspari, W., and Löhe, H.: Weiteres über Heilversuche an geschülstkranken Tieren mittels tumoraffinen Substanzen, Berl. klin Wchnschr. **49:** 1405, 1912.

4. Woglom, W. H.: The Study of Experimental Cancer, New York, 1913, Columbia University Press.

5. Fischer, A.: Embryonale Zellen und Krebszellen, Klin. Wchnschr. **6:** 433, 1927.
   White, A. W. M.: A Study of Sarcoma of the Fowl Produced by Arsenic and Embryonic Pulp, J. Cancer Research **11:** 111, 1927.
   McNeer, G.: Arsenical Keratoses and Epitheliomas, Ann. Surg. **99:** 348, 1934.

6. Hueper, W. C., and Itami, S.: Effects of Neoarsphenamine on Spontaneous Breast Tumors of Mice, Am. J. Cancer **17:** 106, 1933.

7. Cushny, A. R.: Pharmacology and Therapeutics, ed. 9, Philadelphia, 1928, Lea & Febiger.

8. Sollmann, T.: Manual of Pharmacology, ed. 5, Philadelphia, 1936, W. B. Saunders Co.

9. Willberg, M.: Zur Frage nach der Resistenz verschiedenen Tiere geganüber arsen, Biochem. Ztschr. **51:** 231, 1913.

10. Blair-Bell, W.: The Influence of Saturnine Compounds on Cell-growth, With Special Reference to the Treatment of Malignant Neoplasms, Lancet **2:** 1005, 1922.
    Symposium on the Nature of Malignant Neoplasia and Treatment of the Disease With Lead, Brit. M. J. **2:** 919, 1926.
    Some Aspects of the Cancer Problem, London, 1930, Balliere, Tindall & Cox.
    Weller, C. V.: The Blastophoric Effect of Chronic Lead Poisoning, J. M. Research **33:** 271, 1915.
    Stone, W. S., and Craver, L. F.: A Clinical Study of the Colloidal Treatment of Malignant Neoplasms, J. Cancer Research **11:** 204, 1927.
    Lewis, W. C. M.: Some Physico-chemical and Biochemical Aspects of Malignant Neoplasms, Brit. M. J. **2:** 920, 1926.
    Aub, J. C., and Smithwick, R. H.: Lead Treatment of Cancer, New England J. Med. **208:** 310, 1933.

11. Kraemer, W. H.: Cancer Problem, Present and Future, Am. J. Roentgenol. **25:** 793, 1931.
    Preliminary Report on Colloidal Lead Phosphate With Manganese, Am. J. Cancer **15:** 2357, 1931.

12. Hendrick, A. C., and Burton, E. F.: Changes in Bone Tumours After Intravenous Injections of a Colloidal Solution, Canad. M. A. J. **24:** 642, 1931.
    Changes in Bone Sarcoma After Intravenous Injection of a Colloidal Solution of Metallic Arsenic, Canad. M. A. J. **28:** 192, 1933.

13. Vallery-Radot, P., Gilbrin, E., and Gauthier-Villars, P.: Néphrites expérimentales par les sals d'or. Parallélisme entre l'élimination du métal et des lésions histologiques, Ann. de méd. **37**: 145, 1935.
14. Fischer-Wasels, B.: Die Behandlung bösartiger Geschwülste mit Sauerstoff-Kohlensäuregemischen in Verbindung mit Eisen und Eisenfarbstoffen, Frankfurt. Ztschr. f. Path. **39**: 1, 1930.
Experimentelle Grundlagen und folgerungen der Regenerationstheorie der Geschwülstbildung, Klin. Wchnschr. **11**: 1937-1941, 1977-1982, 1932.
Die Entwicklung der Geschwülstzelle, Klin. Wchnschr. **6**: 1025-1029, 1073-1077, 1927.
Die Bedeutung der besonderen Allgemeindisposition des Körpers für die Entstehung der Krebskrankheit und die Mörglichkeiten ihrer Bekämpfung, Strahlentherapie **50**: 5, 1934.
Magot: Versuche mit Phosphatid-Peroxyden bei Tumormäusen,-ratten,-kaninchen (I Mitteilung), Ztschr. f. Krebsforsch. **39**: 219, 1933.
15. Romhányi, J., and Schmidt, M.: Ueber den Eisenhaushalt krebskranker Tiere, Frankfurt. Ztschr. f. Path. **48**: 380, 1935.
Sell, H., and Carls, H.: Der Stoffwechsel der Carcinomzelle unter dem Einfluss oxydationsfördernder und oxydationshemmender Faktoren, Ztschr. f. Krebsforsch. **40**: 416, 1934.
Warburg, O.: Ueber Eisen, den Sauerstoff-übertragenden Bestandteil des Atmungsfermentes, Ber. d. dtsch. chem. Gesellsch. **58**: 1001, 1925.
16. Todd, A. T.: The Selenide Treatment of Cancer, Brit. J. Surg. **21**: 619, 1934.
17. Warburg, O.: Mechanismus der Methylenblauatmung, Ann. Surg. **93**: 205, 1931.
Brooks, Matilda M.: Effect of Methylene Blue on Tumors, Proc. Soc. Exper. Biol. & Med. **30**: 1001, 1933.
18. Fischer-Wasels, B.: Ueber Gasbehandlung bösartiger Geschwülste und kachektischer Zustände, Klin. Wchnschr. **7**: 53-57, 106-110, 153-159, 1928.
Campbell, J. A.: The Use of Gas Mixtures in Cancer Therapy, Cancer Rev. **6**: 289, 1931.
19. Muntwyler, E.: Effect of 1-2-4-dinitrophenol on Oxygen Uptake of Rat Tissue, Proc. Soc. Exper. Biol. & Med. **32**: 1060, 1935.
Krahl, M. E., and Clowes, G. H. A.: Observations on Cellular Oxidative Mechanisms Involved in Dinitrophenol Stimulation of Respiration, Proc. Soc. Exper. Biol. & Med. **32**: 226, 1934.
Terada, B. J., and Tainter, M. L.: Metabolic Response of White Rats to Continued Administration of Dinitrophenol, J. Pharmacol. & Exper. Therap. **54**: 454, 1935.
DeMeio, R. H., and Barron, E. S. G.: Effect of 1-2-4-dinitrophenol on Cellular Respiration, Proc. Soc. Exper. Biol. & Med. **32**: 36, 1934.
McCord, W. M.: The Effect of 2-4 dinitrophenol on the Oxygen Uptake of Kidney and Liver Tissue of the Rat, Am. J. Physiol. **109**: 232, 1934.
20. Coley, W. B.: Late Results of the Treatment of Inoperable Sarcoma by the Mixed Toxins of Erysipelas and Bacillus prodigiosus, Am. J. M. Sc. **131**: 375, 1906.

EXPERIMENTAL UTEROTUBAL INSUFFLATION IN THE RABBIT*

A Preliminary Report

Herbert F. Newman, M.D., New York, N. Y.

FALLOPIAN tube physiology has been investigated since 1925 by means of transuterine insufflation of gas according to the method of Rubin.[1] Essentially, this method consists of recording the fluctuations in pressure of gas insufflated through the uterus and tubes at a relatively constant rate. Feresten and Wimpfheimer[2] have demonstrated the similarity in the response of the rabbit and man to gas insufflation. In studying this field, one is immediately confronted with certain basic problems. How is the pressure curve affected by the type of gas employed, simple repetition of insufflation, rate of gas flow, intra-abdominal pressure, anesthesia, asphyxia, and number of functioning Fallopian tubes connected with the uterus? We have sought preliminary data to answer these questions.

METHOD

Virgin female rabbits, weighing 1 to 2 kg., were used. When anesthetized with nembutal, one grain per kilogram was injected intravenously, providing thorough anesthesia for thirty minutes, and, when the animals awoke, they remained quiet several hours for further investigation. The body temperature did not fall significantly throughout the experiments and no gross changes in cardiac and respiratory rates were observed unless the abdomen was permitted to distend too rapidly with gas.

Most of the experiments were performed by the technique of Feresten and Wimpfheimer.[2] A hypogastric incision was made and the body of one uterus was delivered into the wound. Through a small incision into the uterine wall, about 1 cm. proximal to the cervix, a cannula was directed cephalad and fixed by a ligature which encircled the entire circumference of the uterus to prevent gas from regurgitating through the cervix. The uterus and distal end of the cannula were replaced into the abdomen; the wound was temporarily closed with Allis clamps, and a small opening was made into the epigastrium to permit the free escape of gas.

Some studies were performed on animals with uterine fistulas made according to the technique of Reynolds.[3] Under sterile precautions the vagina was drawn through a small suprapubic incision and divided. The distal portion of the vagina was ligated with silk to prevent peritoneal contamination with urine. The proximal end was drawn through a small stab wound in the midline, cuffed back and sutured to the skin to exteriorize the two cervices. These animals were insufflated without the use of anesthesia, using a clinical

*From the laboratory of the Sydenham Hospital, New York City.
Received for publication, April 2, 1940.

type of cannula with a rubber acorn to prevent the regurgitation of gas. One animal was insufflated daily for two weeks and post-mortem examination failed to reveal any evidence of uterine or pelvic infection.

The cannulae were connected to the usual clinical Grafax Kymographic Insufflation apparatus. This machine employs an anaeroid recording manometer which requires 48 c.c. of gas to record a pressure range of 200 mm. Hg. The clinically available mercurial float type of manometer was found less sensitive, requiring 62 c.c. for a similar pressure range. The insufflating gases were carbon dioxide and oxygen supplied from tanks at 15 pounds pressure. A valve permitted controlled variation in the rate of gas flow. Of necessity, the gas rate cannot be maintained truly constant because the flow rate during insufflation varied according to the pressure differential between the tank source of 760 mm. Hg and the recording kymographic range from 0 to 200 mm. Hg. Constant rates of flow, with pressure measured by an optical manometer, were obtained with fluid rather than with gas by means of a motor-driven syringe. The pressure changes with this medium did not resemble the usual gas insufflation curves and we are at present investigating the mechanism of this discrepancy.

To study the effect of death on insufflation, 30 c.c. of air were injected intravenously. The animal's respirations ceased immediately, and after several convulsive movements the animal became immobile. The heartbeat ceased several minutes later.

EXPERIMENTAL FINDINGS

In the analysis of a typical kymographic tracing, three features were studied: (1) The *primary pressure,* which is attained before gas enters the abdominal cavity, was manifest on the tracing by a characteristic maximal high peak followed by a precipitous fall until a plateau level is reached which is (2) the *general level of pressure;* this is maintained throughout an insufflation and about which (3) *small waves of pressure* fluctuate. These may be superimposed upon larger waves.

Effect of Different Insufflating Gases.—In this investigation the two biologically most important gases, carbon dioxide and oxygen, have been studied in most of the following experiments. Frequent differences have been observed between these gases. The effect of such inert gases as helium and nitrogen and the anesthetic gases are now under investigation.

Effect of Variable Rates of Gas Flow.—In an animal as small as a rabbit with a Fallopian tube luminal diameter of approximately 0.75 mm., small variations in the rate of flow of gas produced changes in the curves. An increase in the rate of flow generally raised the primary pressure level, the general level of insufflating pressure, the size and to a less extent the frequency of the small waves of pressure. At the very slow rates of gas flow some of the small waves of pressure may be submerged due to the low sensitivity of the manometer. Unless indicated, all further experiments were conducted with as constant a rate of flow as could possibly be maintained during a single experiment.

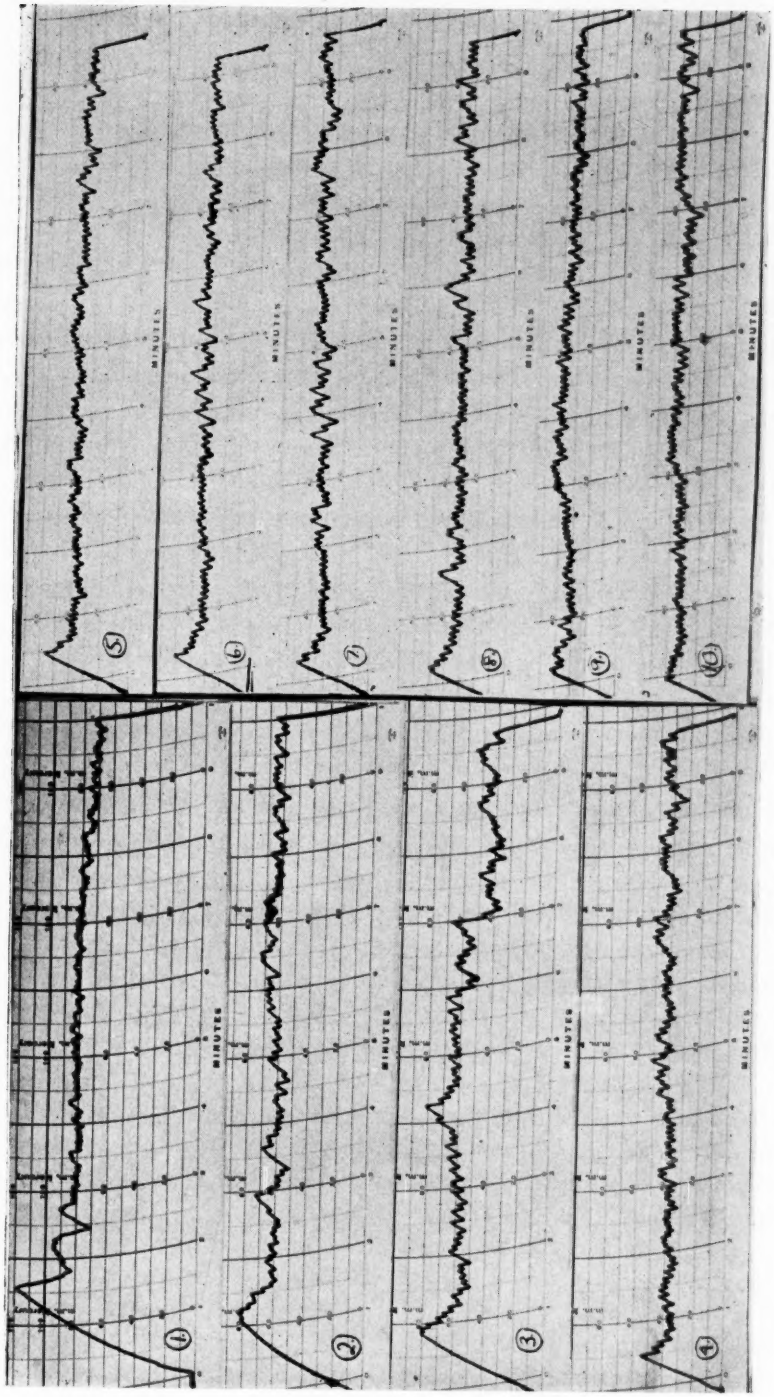


Fig. 1.—Ten consecutive insufflations with oxygen.

Effect of Repeated Consecutive Insufflations.—These animals were insufflated ten times consecutively with each insufflation lasting ten minutes and a rest period of twenty to forty-five seconds between tracings. Of twenty animals, oxygen was used in ten and carbon dioxide in the other ten; slightly different results were obtained.

1. The primary pressure at which gas enters the abdominal cavity generally becomes less on repeated insufflations, reaching the general level of insufflation pressure after about seventy minutes of insufflation. In one animal no gas entered the abdominal cavity on the first insufflation, although a pressure of 200 mm. Hg was reached; on subsequent insufflations the gas entered at progressively lower levels.

2. The general level of pressure also fell on repeated insufflations and remained comparatively stable after seventy minutes.

3. The frequency of the small waves usually decreased if carbon dioxide was used, although in a rare tracing the rate may return to that of the first insufflation. When oxygen was used, the frequency tended to remain more constant (Figs. 1, 2). With both gases the amplitude of the small waves tended to increase (Fig. 2).

4. In one animal, following insufflation of the right uterus for fifty minutes, the left uterus was insufflated. The tracing of the left uterus resembled more that of the first tracing of the right uterus than its fifth (Fig. 3).

Effect of Anesthesia.—Animals were insufflated without anesthesia or under procaine infiltration of the anterior abdominal wall and then reinsufflated after inducing anesthesia to compare the character of the tracings.

1. Nembutal produced no discernible effects in the dosage employed.

2. Ether anesthesia produced diverse effects depending on the gas employed. With carbon dioxide there was a gradual lowering of the general level of pressure and a diminution in the frequency and amplitude of waves (Fig. 4). Often under deep ether narcosis, the manometer recorded zero with no waves. When oxygen was used, the tracing remained normal with the exception of a marked rise in the general level of pressure, even though the animal may be anesthetized to the point of death. This suggests that anesthesia might play a role in the clinical diagnosis of apparent "closed tubes."

Effect of Increased Intra-abdominal Pressure.—Moderate and even marked abdominal distention, if produced gradually (as by obturating the vent in the upper abdomen and permitting the insufflated gas to remain in the abdominal cavity), had no effect upon the curves. The changes produced in intra-abdominal pressure by quiet respiration produced no observable changes in the tracings. However, a sudden increase in intra-abdominal pressure always caused the small waves to fluctuate about a higher general level of pressure. Exciting the unanesthetized animal by pinch stimulation of the ear caused a sudden rise in intra-abdominal pressure and a rise in the general level of pressure recorded on the kymograph. When the abdomen was opened widely to expose the cavity to free atmospheric pressure, repeated stimulation of the ear caused no change in the tracing (Fig. 5).

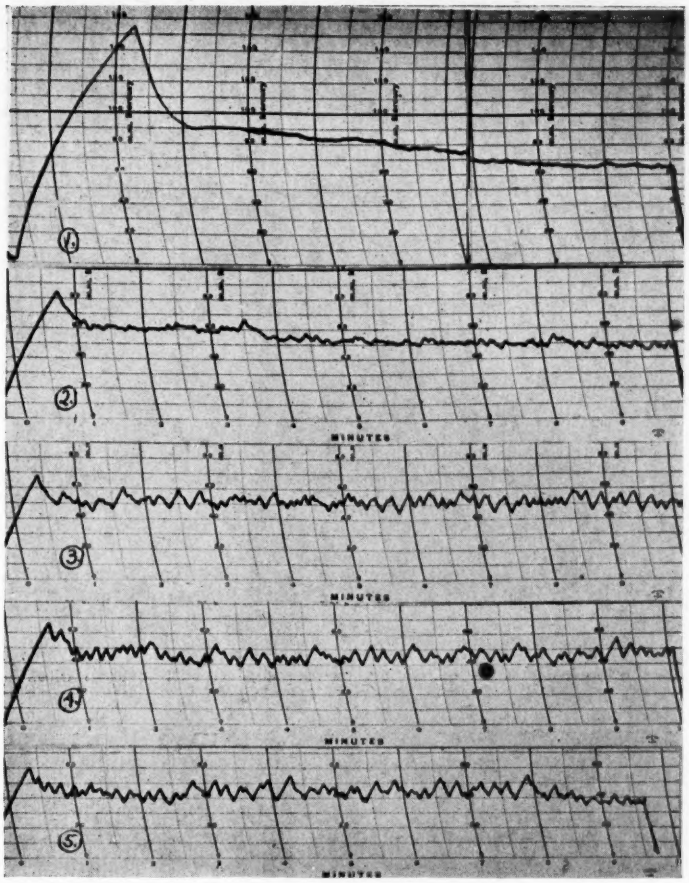


Fig. 2.—Five consecutive insufflations with oxygen, demonstrating the progressive rise in height of the waves.

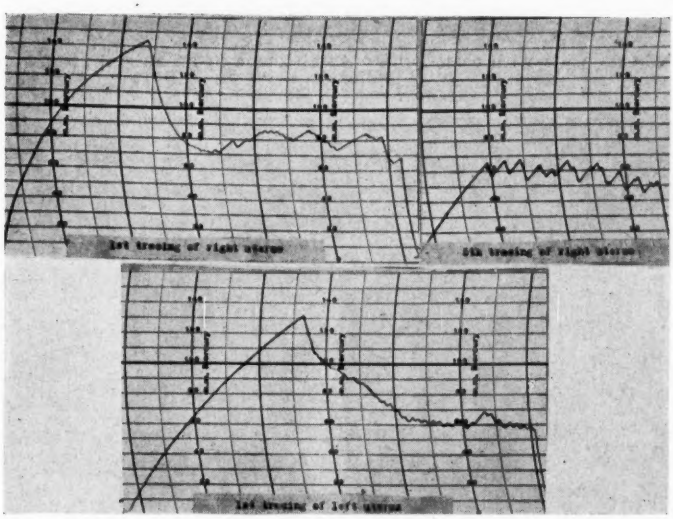


Fig. 3.—Comparison between the first tracing of the left uterus and the first and fifth consecutive tracing of the right uterus.

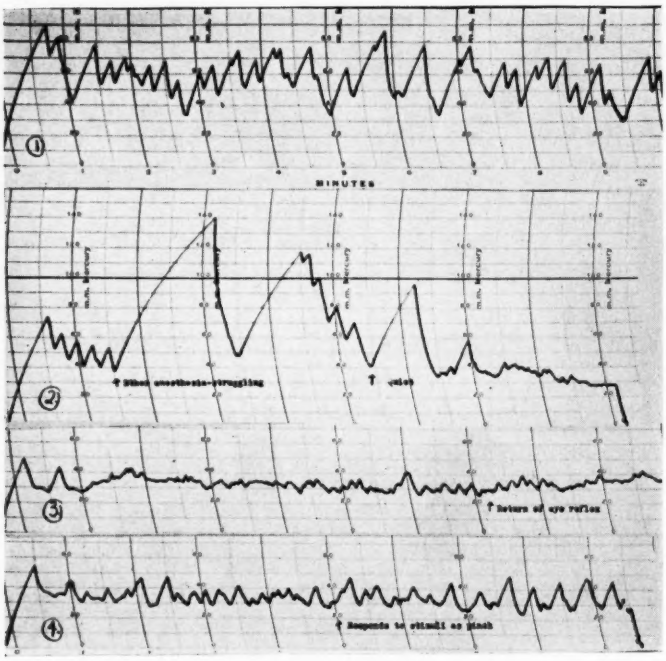


Fig. 4.—Effect of ether anesthesia on an animal insufflated with carbon dioxide with control period of rest.

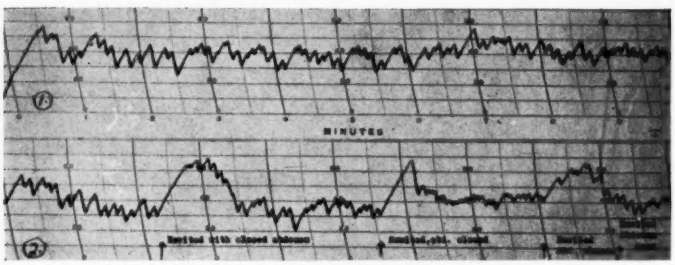


Fig. 5.—Effect of excitation with closed and open abdomen in an animal insufflated with carbon dioxide.

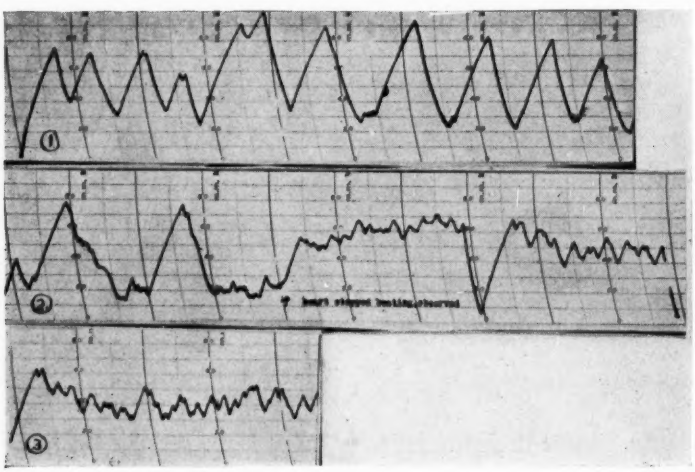


Fig. 6.—Insufflation with oxygen in an animal asphyxiated with excessive nembutal and the change in the tracing upon death of the animal.

Effect of Asphyxia.—In the case of two animals insufflated with carbon dioxide, the dosage of nembutal was apparently excessive, and asphyxia, evidenced by cyanosis and infrequent, shallow respirations, resulted. In these cases very deep and slow undulating waves replaced the usual quick shallow waves of pressure.

An asphyxiated animal, insufflated with oxygen, presented a similar type of curve, but as soon as its heart stopped beating, the tracing returned to a normal variety (Fig. 6). This suggests that an excessive blood carbon dioxide may change the character of the waves.

Insufflation of Dying and Dead Animals.—As soon as respirations of the animals ceased, even though the abdomen was kept open to obviate the effect of increased abdominal pressure induced by the convulsive movements, the general level of insufflating pressure rose to huge heights. When carbon dioxide was used, the curve gradually fell to low levels, with irregular small waves and, on cessation of the heartbeat, the pressure fell to zero and all small waves disappeared (Fig. 7).

When oxygen was used, the characteristic agonal high pressure level was observed and then the pressure fell gradually to a normal level and a normal curve resulted and was maintained for more than twenty-five minutes, although in one animal, the heart had been removed and its lumbar spine transected to produce practically an *in vitro* preparation.

Insufflation with either gas of animals that had been dead for a long time failed to elicit any contractions or any rise in the tracing unless the gas was passed at very rapid rates of flow, when the resultant tracing merely represented the pressure relations produced by the passage of gas through narrow, inanimate channels.

Effect of Simultaneous Insufflation of Both Uteri.—The insufflation of the human uterus with its single cavity and two tubes or the simultaneous insufflation of both uteri in a rabbit suggests two theoretical dynamic possibilities:

1. The resultant tracing is actually produced by gas passing through only one tube.
2. The gas passes through both tubes. This latter possibility brings up the question as to whether the two tubes contract and relax synchronously.

In each of two rabbits both uteri were cannulated and connected by a Y tube to the insufflation apparatus. By appropriate clamps on the tubing each uterus was insufflated in turn and finally both simultaneously. During the bilateral insufflation the gas was observed to bubble from only one tube, the one whose primary pressure level of gas entrance was the lower (Fig. 8).

King[4] with the aid of a double stethoscope reported that in insufflation of human beings, the gas passed either through one or the other tube or through both at once. The pneumoperitoneum produced by insufflation in man has been frequently unilateral. If the gas were to pass through both tubes, the resultant curves in man should exhibit greater variation in the amplitude and frequency of the small waves, with increasing asynchrony of the tubes. Further investigations along this direction are being performed in man.

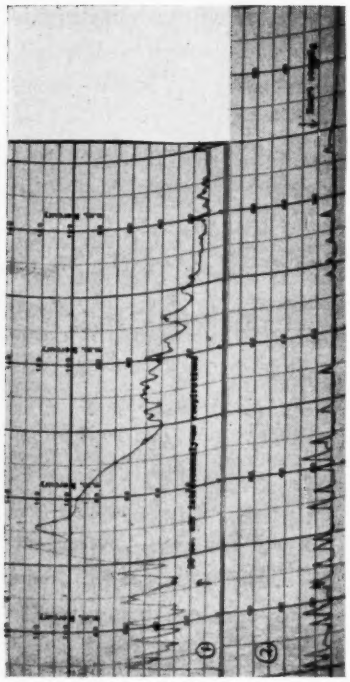


Fig. 7.—Effect of intravenous air upon an animal insufflated with carbon dioxide.

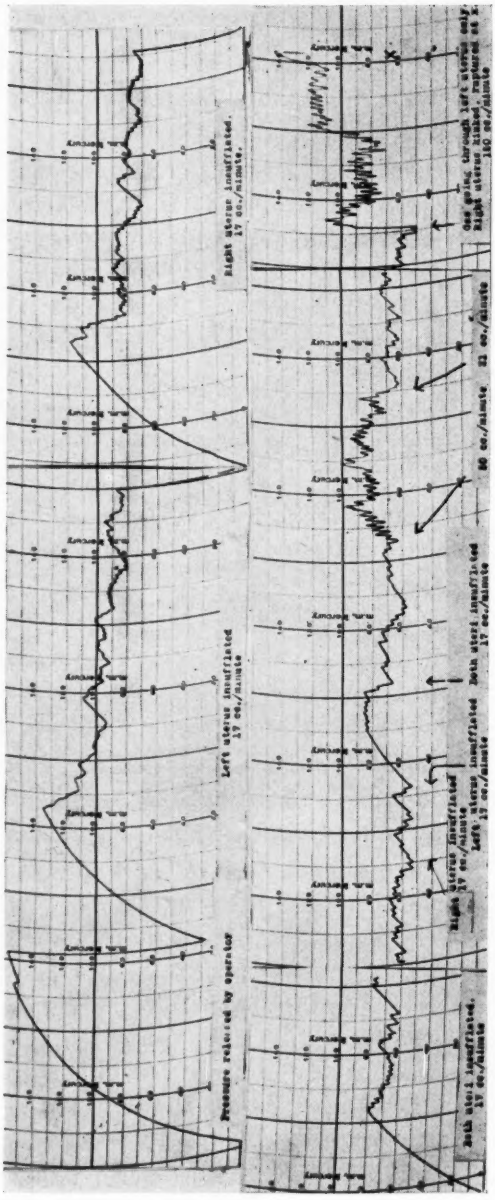


Fig. 8.—Insufflation with carbon dioxide of a bilaterally cannulated animal, demonstrating control tracings for each uterus and subsequent bilateral insufflation.

CONCLUSIONS

1. The type of the curve varies slightly with the kind of insufflating gas.

2. The flow of gas must be kept constant and adequate for the sensitivity of the manometer.

3. Inasmuch as repetition of insufflation modifies the character of the curve, definite criteria must be established to evaluate the curves obtained under experimental conditions as in the investigation of drug and hormonal action.

4. Various anesthetics exert different effects upon the kymographic curves.

I wish to acknowledge the kind advice of Dr. Max Mayer and Dr. Arthur Ginzler and the technical assistance of Mrs. Elizabeth Dreifus.

REFERENCES

1. Rubin, I. C.: Diagnostic Value and Therapeutic Application of Peruterine Insufflation of Fallopian Tubes in Cases of Sterility, Proc. Roy. Soc. Med. **19**: 1, 1925.
2. Feresten, M., and Wimpfheimer, S.: Patency of the Uterotubal Junction of the Rabbit. Experimental Observations With the Aid of CO_2 Insufflation (Rubin Method), Endocrinology **24**: 510, 1939.
3. Reynolds, S.: Studies on the Uterus; Method of Recording Uterine Activity in Chronic Experiments on Unanesthetized Animals, Am. J. Physiol. **92**: 420, 1930.
4. King, G.: Double Stethoscope; Aid in Determining Status of Individual Tubes During Performance of Rubin's Test, Surg., Gynec. & Obst. **63**: 76, 1936.

TREATMENT OF POLYCYTHEMIA VERA WITH LIVER AND CHOLINE HYDROCHLORIDE*

OVID O. MEYER, M.D., AND ETHEL W. THEWLIS, MADISON, WIS.

DAVIS[1] has recently reported that polycythemia induced in dogs by exercise on a treadmill, exposure to reduced pressure in a chamber, or by the administration of cobalt was modified significantly with reduction in the numbers of erythrocytes when whole raw liver or liver extract was administered. In the dogs made polycythemic by cobalt administration or reduced pressure the oral administration of choline hydrochloride, 8 mg. per kilogram in 1 per cent solution per day, also caused diminution in the erythrocyte count after two or three doses.

The mechanism of the production of polycythemia with cobalt is not established with certainty, but it is probably a result of bone marrow stimulus without a physiologic need for this stimulus. The polycythemia of exercise is, of course, but relative, whereas that of reduced pressure is compensatory for a diminished oxygen tension. Therefore, it seemed singular that the same type of therapy could cause reduction in the number of erythrocytes in all three types. Nevertheless, in view of the reported results and the suggestion of Davis that choline may be of benefit in treating polycythemia vera, 5 patients, none of whom had received any previous treatment of any kind for their primary disease, were given either whole raw liver, choline hydrochloride, or both. Raw liver is a good source of choline, containing not less than 1 per cent.[2]

*From the Department of Medicine, University of Wisconsin Medical School, Madison.
This study has been aided in part by a grant from the Wisconsin Alumni Research Foundation.
Received for publication, April 17, 1940.

TABLE I

COMPARISON OF BLOOD FINDINGS BEFORE AND AFTER LIVER AND CHOLINE ADMINISTRATION IN POLYCYTHEMIA VERA

	PATIENT 1 (211,283)		PATIENT 2 (110,726)		PATIENT 3 (211,651)		PATIENT 4 (94,222)			PATIENT 5 (211,985)	
	10/9/39	10/19/39	10/18/39	11/7/39	10/30/39	11/15/39	10/30/39	12/2/39	12/13/39	11/21/39	12/7/39
Hemoglobin (%)	138	130	135	141	168	149	134	125	130	141	157
Erythrocytes	6,175,000	6,245,000	6,258,000	6,595,000	8,388,000	7,263,000	8,645,000	8,250,000	7,700,000	6,478,000	6,838,000
Reticulocytes (%)	0.8	0.9	0.3	1.0	1.0	0.9	2.1	3.0	1.2	0.7	1.0
Hematocrit	60.0	56.7	63.2	66.5	80.7	68.7	66.5	65.4	65.4	69.8	67.6
Plasma, blood volume (c.c.)	2,784		2,300				3,912			2970	
Total blood volume (c.c.)	7,579		6,084				13,321			9349	
Patient's weight (kg.)	63.5		56.3		71.0		72.6			68.2	
	Received 300 Gm. of raw ground liver daily for 10 days		Received 300 Gm. of raw ground liver daily for 19 days		Received 300 Gm. of raw ground liver daily for 14 days		Received 300 Gm. of raw liver daily for 32 days	Received 600 mg. of choline hydrochloride daily for 9 days after initial dose of 300 mg.		Received 550 mg. of choline hydrochloride daily for 12 days after initial dose of 250 mg.	

METHODS

All patients had complete blood counts done; the erythrocyte counts in duplicate, hemoglobin determinations using the Haden-Hausser hemoglobinometer (15.4 Gm. per 100 c.c. equaling 100 per cent); reticulocyte counts on stained smears, the percentage being derived from examining 1,000 erythrocytes; hematocrit determinations using Wintrobe tubes, and blood volume determinations (in four of the five patients as further diagnostic data by the method described by Gibson and Evelyn[3]). The total and differential leucocyte counts are not reported since there were no significant alterations during treatment in any case.

In patient 2, the only one followed as an outpatient, blood studies were done previous to therapy and again at its completion. In the other four patients complete blood counts, reticulocyte counts, and hematocrit determinations were done twice each week throughout each period of observation. For brevity's sake, however, only the data acquired before therapy and at the completion of each period of study are given.

Each of the four patients treated with liver received 100 Gm. of fresh ground raw calf's liver with salt, pepper, and catsup added, or in fruit juice, three times a day. The patients who received choline hydrochloride got 8 mg. per kilogram body weight in 1 per cent solution. The five patients were males and their respective ages were 64, 51, 54, 20, and 59. They all took choline hydrochloride and liver with little or no difficulty.

DISCUSSION

The results appear to be clearly negative in four patients as far as reduction of erythrocytes or hemoglobin is concerned, for there was no significant change in any patient while undergoing liver or choline therapy. In patient 3, who received liver therapy, there was a decrease in hemoglobin and erythrocytes, and in the hematocrit reading. This diminution had its inception on the tenth day of treatment and was of the same degree on the fourteenth day. During the period of observation, before a fall in the blood levels was demonstrable, a wet granular ulcerative lesion on the leg was present. Coincident with changes in the blood, complete healing occurred. Whether hemoconcentration may have played a part in explanation of the previous very high hemoglobin and erythrocyte levels is problematical. Attempts at determining the blood volume preceding therapy were unsuccessful since pronounced viscosity of the blood and prompt coagulation interfered with collection of blood samples.

Marshall[4] has reported that cobalt polycythemia in rats is modified with reduction in the erythrocyte count following liver extract or ventriculin administration but not after feeding fresh whole liver. Stephan[5] has reported that whole fresh liver was of clinical value in treating the polycythemia symptomatic of a variety of conditions, but Major[6] noted no benefit from liver extract administration in three cases of polycythemia vera. We have treated no case of polycythemia with liver extract. Ogawa[7] successfully treated one case of polycythemia vera with oral and parenteral liver substance, but the polycythemia recurred with the cessation of therapy.

Our results with liver therapy in polycythemia vera are not absolutely conclusive for during therapy one patient showed a decrease in erythrocytes and

hemoglobin, although there was an extenuating circumstance in this case. In the other two patients treated with raw calf's liver alone, in the one patient treated with liver and later with choline hydrochloride, and in the one patient treated with choline alone there were no significant changes in the blood levels. It is our interpretation that the administration of raw calf's liver or choline hydrochloride is probably without value in the treatment of polycythemia vera.

We wish to acknowledge the kind cooperation of Dr. Marian S. Kimble who did the blood volume determinations.

REFERENCES

1. Davis, J. E.: Reduction of Experimental Polycythemias by Liver Administration, Am. J. Physiol. **122**: 397, 1938; Depression of Experimental Polycythemias by Choline Hydrochloride or Liver Administration, Ibid. **127**: 322, 1939; Depression of Polycythemia by Choline Hydrochloride, Proc. Soc. Exper. Biol. & Med. **40**: 445, 1939.
2. Jacobs, H. R.: On Nature of Antipernicious Anemia Principle, Search for Nitrogenous Bases Isolation of Choline, J. LAB. & CLIN. MED. **24**: 128, 1938.
3. Gibson, J. G., and Evelyn K. A.: Clinical Studies of Blood Volume; Adaption of Method to Photoelectric Microcolorimeter, J. Clin. Investigation **153**: 17, 1938.
4. Marshall, L. H.: Antianemic Treatment in Experimental Polycythemia, Am. J. Physiol. **114**: 194, 1935.
5. Stephan, R.: Symptomatische Polyglobulie and Lebersubstanz-Therapie, Ein Beitrage zur Pathogenese der perinziösen Anämie, Klin. Wchnschr. **9**: 1068, 1930.
6. Major, R. H.: Effect of Liver Extract Upon Polycythemia Vera, J. LAB. & CLIN. MED. **24**: 65, 1938.
7. Ogawa, J.: Über den Einfluss der Lebersubstanz auf Polycythaemia vera, zugleich ein Beitrag zur Milzdiättherapie, Jap. J. M. Sc. **2**: 267, 1932.

FURTHER STUDIES ON THE EFFECT OF MASSIVE INTRAVENOUS INFUSIONS IN NARCOTIC POISONING*

THEODORE KOPPANYI, PH.D., AND REGINALD A. CUTTING, PH.D., M.D.
WASHINGTON, D. C.

IN A PREVIOUS communication[1] we described the effects of massive intravenous infusions of isotonic glucose or saline solutions, in volumes varying from one-fourth to three-fourths the body weight, on the duration of barbiturate narcosis. It was found that infusions of such volumes of fluid accelerated the time of recovery of anesthetized animals and prevented death in those receiving fatal or several fatal doses of these drugs. It was suggested that the method of treatment with massive intravenous infusions may similarly prove antidotal to all slowly acting neurotoxic drugs, particularly central depressants. The present report deals with the experimental exploration of this hypothesis.

Dogs were used in all the experiments. For the technique of administering massive intravenous infusions reference should be made to the preceding paper.[1] A solution of 1 per cent sodium chloride was used for infusions in this series. It should be emphasized in this connection that care in the technique of the preparation of solutions to be infused in quantities such as we have used

*From the Departments of Pharmacology and Materia Medica and Physiology, Georgetown University School of Medicine.
Received for publication, April 10, 1940.

is essential. This is to avoid the possibility of contamination with pyrogenic agents and other harmful contaminants possibly present in water. Scrupulous asepsis should be observed throughout, and the rates of infusion should be carefully controlled. One of the principal mechanisms likely to cause fatalities from massive infusions is insufficient diuresis. It is the retained fluid, rather than the total amount infused, which is critical. Animals withstand more rapid rates of infusion and larger total quantities of solution when the kidneys eliminate urine in considerable quantities. The effect of massive infusions was tested on the course of poisoning by oral, hypodermic, or intravenous fatal doses of the following substances: methyl alcohol, ethyl alcohol, chloral hydrate, sodium bromide, and potassium arsenite. Whenever oral doses were given, the animals also received a preliminary injection of 10 mg. per kilogram of body weight of morphine sulfate* intramuscularly to abolish the vomiting reflex and thus insure retention of the dose. Inasmuch as morphine is a well-known synergist to all aliphatic narcotics, one may assume that morphine enhanced the toxicity of the orally administered drugs.

Many animals not listed in the following discussion and tabular protocols were used during the course of the experiments. For every dose of methanol, ethanol, chloral hydrate, and sodium bromide here reported, doses of similar magnitude were administered to two or more animals which died before infusion could be instituted, and others receiving such doses died during the infusions. All these may be regarded as constituting control experiments and as supporting the observations of other investigators that all doses used were considerably in excess of the ordinary fatal dose.

Methanol.—Methanol was administered by mouth in two animals thirty minutes following a preliminary injection of morphine to control vomiting. Absolute methanol in doses of 20 c.c. diluted with water was given to each animal. No regurgitation or vomiting occurred. The intravenous infusion was started as soon as the animals' righting reflexes were lost. Subsequently the animals showed complete anesthesia and muscular relaxation. Following the infusion the animals were kept in warm metabolism cages until recovery was complete. Both animals recovered. The index of recovery was the ability of the animal to stand unsupported. The average oral fatal dose of methanol in dogs is 10 c.c. Thus these animals survived two fatal doses.

One animal received 10 c.c. of methanol dissolved in 1 per cent sodium chloride solution by slow intravenous infusion over a period of forty-three minutes, becoming anesthetic after half of the dose had been injected. This dog recovered in about thirty-six hours. The urine excreted by this animal following injection of the alcohol was of a deep red color, evidently the result of hemolysis.

No evidence of blindness was seen in any of these animals though they were observed for several weeks after recovery.

Ethanol.—Ethanol was administered to two animals orally and to one animal intravenously, as described for methanol. One animal, receiving 15 c.c.

*All doses are expressed in terms of milligram, gram, or cubic centimeter per kilogram of body weight. To avoid repetition the phrase "per kilogram of body weight" will hereafter be omitted.

of ethanol by mouth, recovered, and one, receiving 20 c.c. by mouth, died. A dog receiving 12 c.c. intravenously recovered. The average oral fatal dose of ethanol for dogs is 9.6 c.c.

Chloral Hydrate.—Chloral hydrate was administered in aqueous solution to one animal subcutaneously and to two animals intravenously. The technique employed was the same as that described for methanol. The mean subcutaneous fatal dose of chloral hydrate is 600 mg., whereas 500 mg. are fatal by intravenous injection. Inasmuch as considerably more than this amount was administered, the results may be regarded as significant.

Sodium Bromide.—Sodium bromide in doses of 5.0 Gm. and 7.5 Gm. was administered to each of two dogs in the following manner: The animals were immobilized in plaster of Paris bandages. Under local anesthesia the external jugular vein was exposed and cannulated. An intravenous infusion of 1.5 per cent sodium bromide solution (isotonic) was then given in predetermined dosage. Beyond this point 1 per cent sodium chloride solution was substituted. Although both animals recovered, they showed somnolence for several days and shed quantities of hair; portions of their skin became practically denuded, and deep ulcers developed on various parts of their bodies, particularly over bony prominences. The mean fatal dose of sodium bromide is 3 Gm.

Potassium Arsenite.—Each of five animals was given 2 c.c. of potassium arsenite solution (U.S.P. XI) by mouth. Two of these animals received massive intravenous infusions, the remaining three served as controls. All five animals died. All animals, including those which received massive infusions, lapsed into coma, developed muscular spasms, and died within about the same time. It is concluded, therefore, that massive intravenous infusions had no effect on the course of acute arsenical poisoning. Table I summarizes the results of all experiments previously described.

TABLE I

EFFECT OF MASSIVE INTRAVENOUS INFUSIONS WITH 1 PER CENT SODIUM CHLORIDE SOLUTION ON THE FATE OF DOGS* POISONED WITH FATAL DOSES OF METHANOL, ETHANOL, CHLORAL HYDRATE, SODIUM BROMIDE, AND POTASSIUM ARSENITE

WEIGHT (KG.)	DRUG	ROUTE OF ADMINISTRATION	DOSE PER KG. (C.C. OR GM.)	AMOUNT OF FLUID INJECTED		TIME REQUIRED FOR INFUSION (HR.)	RESULTS
				TOTAL (C.C.)	% OF BODY WT.		
7.5	Methanol	Oral	20	3,000	40.0	3	Recovered in 72 hours
8.2	Methanol	Oral	20	4,800	53.5	5½	Recovered in 96 hours
16.3	Methanol	Vein	10	5,375	32.8	12	Recovered in 36 hours
15.1	Ethanol	Oral	15	6,600	43.7	4	Recovered in 48 hours
8.8	Ethanol	Oral	20	2,700	30.7	2½	Died in 3 hours
9.25	Ethanol	Vein	12	2,700	29.2	2	Recovered in 24 hours
17.45	Chloral hydrate	Subcutaneous	1	7,500	43.0	2	Recovered in 12 hours
16.6	Chloral hydrate	Vein	0.75	6,025	36.4	4	Recovered in 12 hours
8.5	Chloral hydrate	Vein	1	4,000	47.0	4	Died in 5 hours
5.4	Sodium bromide	Vein	5	2,600	48.1	4	Recovered in 24 hours
11.8	Sodium bromide	Vein	7.5	10,200	92.0	48	Recovered in 72 hours
8.0	Solution of potassium arsenite	Oral	2	5,100	63.7	5	Died in 7 hours
4.55	Solution of potassium arsenite	Oral	2	2,700	59.4	3	Died in 12 hours

*These animals excreted in the urine from 20 to 30 per cent of the infused fluid during the course of the infusion, and from 40 to 90 per cent within twenty-four hours.

COMMENT

In our previous communication[1] we discussed the probable mechanism of the antidotal action of massive intravenous infusions in acute barbiturate poisoning. We expressed the opinion that this action is dependent upon the ability of the infused solution to take up a part of the drug, which otherwise would accumulate in toxic concentration in nervous tissues, and to distribute it among the tissues of the body not specifically affected by the drug. When the infused fluids are excreted by the kidneys, the urine contains the neurotoxic poisons in high concentrations, higher than exist in any of the tissues of the body. These considerations apply to neurotoxic poisons because the central nervous system, in contrast to other tissues, does not become hydrated as a result of massive infusions.[2] The experiments herein described show that the results obtained with barbiturates can be duplicated in the case of three other aliphatic narcotics and sodium bromide, but not in inorganic arsenical poisoning, because arsenic is not primarily a neurotoxic agent and behaves rather as a general protoplasmic poison.

In conclusion, we feel justified in repeating our recommendation for the clinical use of large intravenous infusions in the treatment of alcohol comas, and acute chloral hydrate and bromide poisonings, though not necessarily in the extremely energetic form used in these experiments. Massive infusions should prove less effective or probably not effective in poisoning with general protoplasmic toxins. This treatment is not proposed as a replacement for the rapidly acting analeptics in narcotic poisoning, but rather as an adjuvant. It may be of particular value in cases in which patients do not show a satisfactory response to the analeptic treatment.

REFERENCES

1. Cutting, R. A., and Koppanyi, T.: The Effect of Massive Intravenous Infusions on the Course of Barbiturate Narcosis, Arch. internat. de pharmacodyn. et de thérap. 60: 395, 1938.
2. Cutting, R. A., Lands, A. M., and Larson, P. S.: Distribution and Excretion of Water and Chlorides After Massive Saline Infusions, Arch. Surg. 36: 586, 1938.

CLINICAL CHEMISTRY

PLASMA VOLUME AND PLASMA PROTEIN CONCENTRATION AFTER SEVERE HEMORRHAGE*

D. Bailey Calvin, M.A., Ph.D., Galveston, Texas

RESEARCHES recently reported[1, 2] have suggested a possible increase in total circulating plasma protein following shortly after severe hemorrhage. From work previously reported from this laboratory, it is known[3, 4] that in hydremic plethora extra protein may enter the vascular system from some source in the body, possibly the liver.[5] Similar changes have been observed with increased plasma volume, following the use of diuretics in the treatment of the edema of circulatory failure and cardiac decompensation.[6] The present report deals with this problem of protein mobilization in hemorrhage, employing the newer techniques for plasma volume determination.

EXPERIMENTAL

Large dogs were used. Three types of preparation were employed. (A) Dogs without food or water for forty-eight hours prior to hemorrhage. (B) Dogs allowed water but no food twenty-four hours prior to hemorrhage, then given 500 ml. of 0.7 per cent sodium chloride solution by mouth one hour prior to hemorrhage. (C) Dogs allowed water but no food twenty-four hours prior to hemorrhage, with injection into the jugular vein of a volume of 0.9 per cent sodium chloride equivalent to the volume of whole blood withdrawn, immediately after withdrawal.

The hemorrhage from the carotid artery was 25 per cent of the dog's blood volume (as previously determined) within five minutes.

Plasma volumes were determined by the Gibson and Evelyn[7] techniques; total proteins by the Kjeldahl method; hemoglobins according to Cohen and Smith;[8] albumins by the Howe,[9] and later by the Campbell and Hanna[10] methods. Mayo 6 ml. tubes at 3,000 r.p.m. for thirty minutes were used for cell volumes.

RESULTS

A total of 11 dogs was used, 4 in group A, 4 in group B, and 3 in group C. Typical data are presented graphically in Figs. 1 *A* and *B*, and Fig. 2, *C*.

The animals in group A were very dehydrated. This condition is reflected in the posthemorrhage blood picture. There is a slow fall in hemoglobin and per cent of plasma protein. In contrast to these changes, there is a slow rise in plasma volume, and a similar but slightly faster rise in total circulating

*From the Department of Biological Chemistry, University of Texas School of Medicine, Galveston.

Received for publication, July 27, 1940.

plasma protein (plasma volume/100 × Gm. protein per 100 ml.). This extra fluid and protein must come from the tissue (or lymph), the protein entering in solution with the water. The changes observed in group A were never so great as for the conditions to be described in B and C, ranging from about 10 to 20 per cent replacement of the total protein removed in hemorrhage.

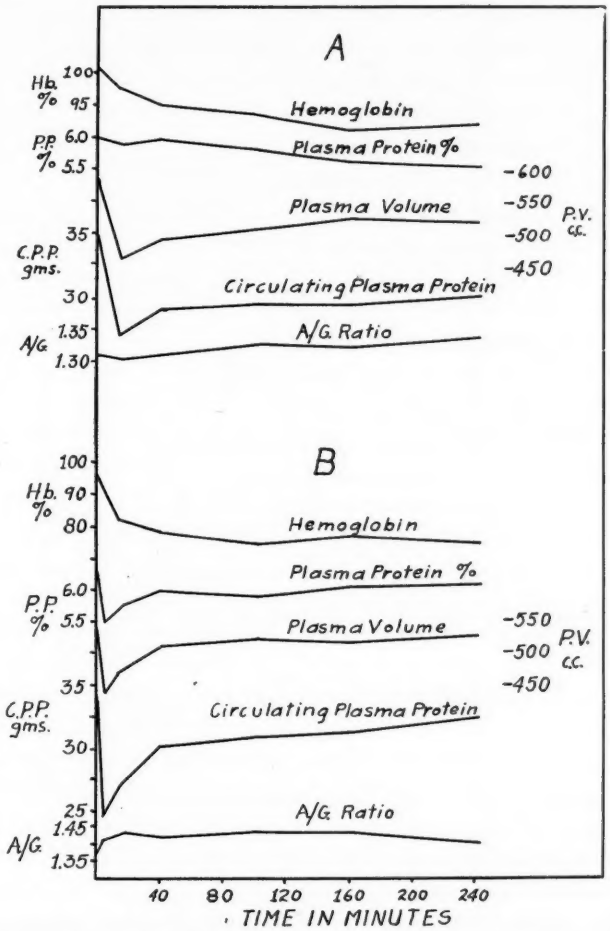


Fig. 1.—*A*, Hemorrhage after forty-eight hours food and water fast. *B*, Hemorrhage after twenty-four hours water fast; 500 ml. of 0.7 per cent sodium chloride per os one hour prior to hemorrhage.

In group B the dogs were somewhat dehydrated, but in fair water balance. To this adequate body fluid was added 500 ml. of saline solution, given by mouth, one hour prior to hemorrhage. The one-hour period was selected because of a previous finding[3] that injected saline reaches a point of maximum storage in the tissues from sixty to one hundred minutes after intravenous administration. Very little or no diuresis occurred. Thus the body fluids available for posthemorrhage blood volume compensation were at a maximum.

The greater availability of fluid is amply demonstrated in Fig. 1 *B*. The hemoglobin drops more sharply than in group A. This is also true of plasma protein concentration (per cent). The plasma volume, on the other hand, does not fall off so rapidly, indicating a very rapid inflow of fluid from the inter-

stitial spaces. As in group A, so also here to a more marked degree, this inflowing fluid brings with it considerable amounts of protein, as measured in terms of total circulating plasma protein.

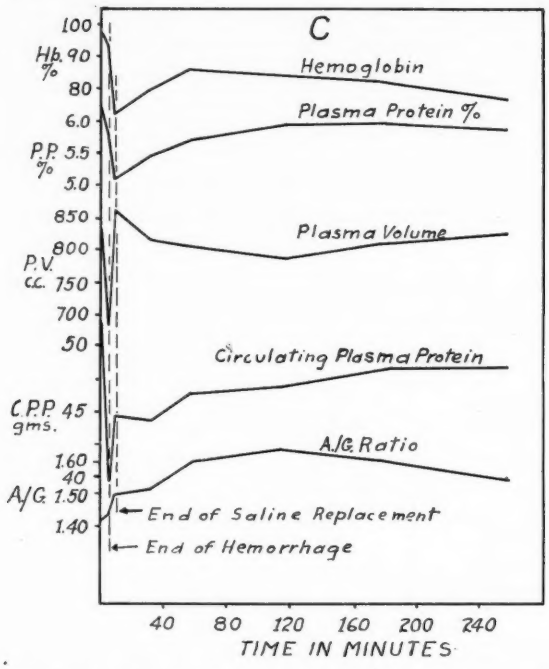


Fig. 2.—*C*, Hemorrhage after twenty-four hours water fast. A volume of 0.9 per cent sodium chloride equivalent to the volume of blood removed is injected into the jugular vein immediately after hemorrhage.

The animals in group C were in similar fluid balance to those in B, prior to per os saline administration. Immediately after hemorrhage was complete, a volume of 0.9 per cent sodium chloride, equivalent to the volume of blood removed, was injected rapidly into the jugular vein. The initial rapid changes prior to injection of saline were similar to, but somewhat less than, those in group B. After saline injection, however, marked falls in hemoglobin and in per cent plasma protein occurred.

The plasma volume changes were of much interest. From the low value after hemorrhage the volume actually rose above the normal level. However, this condition held only momentarily. As was to be expected from previous data on hemorrhage control,[11] the injected saline left the vascular system rapidly and to a considerable extent, resulting in a fall in plasma volume. This condition obtained for upward of an hour, at which time the plasma volume showed a tendency to rise.

During the whole of this period, and more markedly in the first few minutes, extra protein entered the vascular system, as evidenced by the initially rapid, then gradual, rise in total circulating plasma protein.

In all groups, the albumin : globulin ratio rose following hemorrhage.

DISCUSSION

The results here presented must not be confused with those obtained in *acute* hemorrhage or after plasmapheresis. The degree of hemorrhage used (25 per

cent of the determined blood volume) was not sufficiently severe to upset physiologic equilibrium in the dog. An effort was made to avoid gross evidence of shock. No animals were lost as a result of the procedure. There was, however, some blanching of the buccal membranes, and the heart rate was increased to some extent. Blood pressures were not measured.

Thus the animals were able to affect a rather satisfactory recovery by normal, rapid physiologic response. It is remarkable to note the degree to which this can be accomplished. In one dog, group C, 14.7 Gm. of plasma protein were removed. Within thirty minutes 6.5 Gm. of this had been replaced, and at the end of four hours more than 50 per cent of the plasma protein loss had been taken care of by tissue contribution of storage protein. These values are in line with others obtained in dogs after injections of hypertonic solutions,[4] where an increase as great as 20 per cent has been observed to occur along with the resultant increase in plasma volume.

The albumin:globulin ratio is of significant interest. This ratio rose in all cases following hemorrhage. The extent of the rise depended upon the degree of total compensation affected in plasma protein, being higher when the increase in total circulating plasma protein was greater. This would seem to indicate, therefore, that the protein entering the vascular system under this condition of stress was composed mainly of albumin. These results are similar to those for hydremic plethora[4] and those following edema reduction by diuretics.[6]

The exact mechanisms for the changes noted are hard to evaluate. The lymph certainly may serve as one source of this protein and fluid increase. Indeed, evidence has been given for an increased lymphatic flow following similar conditions. The *rate* of change observed, however, would seem to make it necessary to assume some degree of backflow through the capillaries both of fluid and protein in order to explain the experimental results. Recently, Drinker[12] gave some support to this idea.

The matter of source of protein for the observed increases is not difficult to explain. The liver is known to produce at least part of the protein necessary for plasma protein regeneration. Considering the size of this organ, the slow rate of circulation through it, and the relatively small demand on the whole body made by the replacement of 15 to 20 Gm. of plasma protein, the experimental findings are not untenable. Furthermore, when consideration is given to the total mass of protein in the body, this small amount might easily be accounted for physiologically without being detectable by analytic chemical methods.

It is logical to assume, therefore, on the strength of the data presented, that the body is capable of supplying, rapidly, considerable amounts of protein for the replacement of losses due to hemorrhage, so long as actual gross shock does not intervene. Replacement of fluid depends upon available interstitial supply of water and salts for this purpose.

SUMMARY AND CONCLUSIONS

1. Following subacute hemorrhage in dogs, there is a compensatory movement of fluid from the tissues to the plasma.

2. The degree of compensation depends, in large measure, on the water balance of the body prior to hemorrhage.

3. Proteins enter the vascular system along with the fluid.
4. The protein entering the vascular system is primarily albumin.

REFERENCES

1. Hoitink, A. W. J. H.: Colloid-Osmotic Pressure of Plasma and Composition of Blood After Acute Haemorrhage and After Haemorrhage Followed by Intravenous Injection of 0.9% NaCl Solution, Arch. néerl. de physiol. **24**: 169, 1939.
Idem: Colloid-Osmotic Pressure of Plasma and Composition of Blood After Intravenous Injection of Gum-Salt-Solution in Hemorrhage, Arch. néerl. de physiol. **24**: 194, 1939.
2. Elman, R.: Acute Hypoproteinemia Following Single Severe Hemorrhage in Fasting Dogs, Am. J. Physiol. **128**: 332, 1940.
3. Calvin, D. Bailey, Smith, A. H., and Mendel, L. B.: A Study of Blood Volume Regulation and Blood Composition in Experimental Hydremia, Am. J. Physiol. **105**: 135, 1933.
4. Calvin, D. Bailey: Changes in Albumin:Globulin Ratios Following Intravenous Saline Injections, Am. J. Physiol. **129**: 327, 1940.
5. Madden, S. C., and Whipple, G. H.: Plasma Proteins: Their Source, Production and Utilization, Physiol. Rev. **20**: 194, 1940.
6. Calvin, D. Bailey, Decherd, G., and Herrmann, G.: Response of Plasma Volume to Diuretics, Proc. Soc. Exper. Biol. & Med. **44**: 529, 1940.
Idem: Plasma Protein Shifts During Diuresis, Proc. Soc. Exper. Biol. & Med. **44**: 578, 1940.
7. Gibson, J. G., Jr., and Evelyn, K. A.: Clinical Studies of Blood Volume; Adaptation of Method to Photoelectric Microcolorimeter, J. Clin. Investigation **17**: 153, 1938.
8. Cohen, B., and Smith, A. H.: The Colorimetric Determination of Hemoglobin, J. Biol. Chem. **39**: 489, 1919.
9. Howe, P. E.: The Use of Sodium Sulphate as the Globulin Precipitant in the Determination of Proteins in Blood, J. Biol. Chem. **49**: 93, 1921.
10. Campbell, W. R., and Hanna, M. I.: The Albumin, Globulins and Fibrinogen of Serum and Plasma, J. Biol. Chem. **119**: 15, 1937.
11. Erlanger, J.: Blood Volume and Its Regulation, Physiol. Rev. **1**: 177, 1921.
12. Drinker, C. K.: Quoted from address, New Orleans, March, 1940.

PHENOLPHTHALEIN STUDIES: THE RATIO OF FREE TO TOTAL PHENOLPHTHALEIN IN THE URINE*

Its Influence Upon the "Woldman Test"

F. Steigmann, M.S., M.D., and J. M. Dyniewicz, Ph.C., Chicago, Ill.

IN OUR preliminary report[1] which was based on 200 cases (34 normal persons and 166 diseased patients) we found Woldman's test positive in 41 per cent of the normal individuals, in 78.5 per cent of the gastrointestinal cases, and in 82.7 per cent of other types of disease, including jaundice. Our result of positive Woldman tests in 41 per cent of the normal persons is perhaps somewhat higher than the percentage in similar cases obtained by others. On the other hand, however, we also obtained higher positive results in gastrointestinal cases (78.5 per cent) as compared to results by others, e.g., 72 per cent. The high incidence of positive results in our series may be attributed to meticulous interpretation of color changes.

*From the Departments of Pharmacology and Therapeutics and of Medicine, University of Illinois College of Medicine, and the Cook County Hospital, Department of Medicine, Chicago.

Assisted by a grant from Phenolphthalein Research, Inc., under the direction of Dr. Bernard Fantus.

Received for publication, March 29, 1940.

Our original observation that the urinary elimination of free phenolphthalein is no test for gastrointestinal ulceration has been confirmed not only by further studies in our laboratories but also by reports from various other clinics. Thus Kremer and others[2] in a study of 137 cases conclude that the Woldman test may be valuable as an adjunct in diagnosis, but that more must be learned of the physiology involved before it can be considered reliable as a simple test for the determination of gastrointestinal lesions.

Suttenfield,[3] from his study of 94 cases, concludes that the proposed phenolphthalein test is of no value in differentiating gastrointestinal disease from other diseases, nor organic from functional intestinal diseases.

In a study of 203 patients Banks[4] found the test negative in one-fourth of his cases with proved gastrointestinal disease, and positive in one-sixth of the controls. He believes that the test has too wide a range of error to be considered reliable in the diagnosis of gastrointestinal disease.

In his series of 105 individuals Notkin[5] obtained negative Woldman tests in over 30 per cent of his cases with active gastrointestinal lesions. He also discovered variations from negative to strongly positive in the same patients when the test was repeated at appropriate intervals. He concludes, therefore, that the Woldman phenolphthalein test does not constitute a test for the presence of gastrointestinal lesions.

LeVine,[6] from a study of 115 cases, concludes that the phenolphthalein test cannot be relied on to prove or disprove the presence of a lesion in the mucous membrane of the gastrointestinal tract.

Slutzky and Wilhelmj,[7] from experiments on dogs, believe that the variability of their results would seem definitely to exclude the phenolphthalein test as a test for determining the presence of ulceration of the gastrointestinal tract.

Rowland,[8] however, believes that not enough observations have been done to negate the test completely. In his defense of this test, however, appear certain concepts of phenolphthalein absorption, conjugation, and elimination which are not in accordance with our observations on these facts gained through a study on hundreds of cases during a period of four years. This paper has, therefore, been written with the idea of clarifying some of the more important data about the fate of phenolphthalein in the body: data which may indirectly help in the better understanding of the facts underlying and influencing the Woldman test.

RAPIDITY OF ABSORPTION

Woldman's test is based upon the thesis that, if there is an ulcer in stomach or bowel, ingestion of phenolphthalein in rapidly absorbable form (alcoholic solution) will permit the finding of free phenolphthalein in the urine because the break in the mucosa allows phenolphthalein to enter the circulation more promptly. It is obvious that we cannot compare the results of the quantitative studies done previously by Fantus and Dyniewicz,[9] and quoted by Rowland, with the results now being discussed. There is a much greater and prompter elimination of phenolphthalein after the alcoholic dose of phenolphthalein than after the same or even larger doses given in capsule form. The time factor in the two studies was also different. In the "Woldman's test" studies the speed of

elimination is measured at two-hour intervals for ten hours, while in the Fantus and Dyniewicz studies interest was centered in the total elimination as shown by collections of twenty-four-hour specimens.

The previous work on phenolphthalein elimination was done after ingestion of doses ranging from 0.10 Gm. to 0.25 Gm. in capsule form. The total elimination was studied for as long as six days. The elimination of from 1.41 to 19.7 per cent was for all doses. That many factors influence the elimination of phenolphthalein in the urine was recognized at that time and set forth in the article, e.g., "the larger the dose the smaller the percentage of the elimination" and "the greater the laxative action the less the elimination." We now are aware of additional causes which influence the amount of phenolphthalein eliminated in the urine, e.g., low elimination in kidney disease and high elimination in "yellow" block (jaundice) and in "white" block (sepsis). There is, of course, a great difference in phenolphthalein elimination when the drug is taken in alcoholic solution or in capsule form. When 100 mg. of phenolphthalein in an alcoholic solution are taken on an empty stomach, we obtain on the average 24 mg. per cent of total phenolphthalein in the stool and 38 and more per cent in the urine. After the ingestion of 300 mg. of phenolphthalein in a capsule, an average of 75.5 per cent of the dose was found in the stool and only 8.9 per cent on the average in the urine. The reason for the apparent discrepancy between our present results and some of the data in our former reports is, therefore, plain.

TABLE I

NO. OF SPECIMENS	FREE PHENOLPHTHALEIN PRESENT IN URINE (MG. %)	AVG. CONC. OF CONJUGATED PHENOLPHTHALEIN PRESENT IN URINE (MG. %)
162	0.0	1.31
63	0.05	2.55
62	0.15	5.0
27	0.25	7.4
12	0.35	7.6
12	0.45	9.6
29	More than 0.45	11.0

When one desires to favor absorption of phenolphthalein and does not give it for cathartic action, its administration by the method suggested by Woldman, e.g., in alcoholic solution on an empty stomach, eliminates at least three variable factors: the phenolphthalein is not dependent for solution upon the secretions of the intestines; a more uniform and more rapid absorption occurs, since there is no food interference; and the alcoholic vehicle helps absorption from the stomach. Furthermore, by studying the phenolphthalein elimination at two-hour instead of twenty-four-hour intervals, the amount of phenolphthalein in the urine is much less likely to be influenced by the extent of laxative action, which usually occurs after six hours.

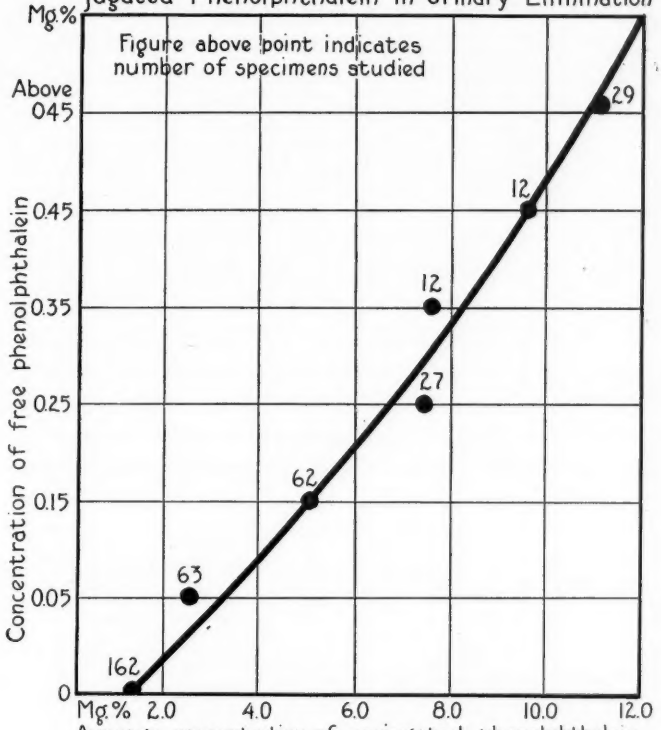
The ten-hour elimination after the alcoholic dose of phenolphthalein frequently exceeds the amount eliminated in six days after the same dose given in capsule form, and consequently the finding of free phenolphthalein is that much more common, as this depends upon two factors: the quantity of total phenolphthalein in the urine, and the bacterial decomposition of the conjugated phenolphthalein.

RELATION OF FREE TO CONJUGATED PHENOLPHTHALEIN

The amount of free phenolphthalein found in the urine is largely influenced by the quantity of conjugated phenolphthalein present (as is shown in Table I and Graph 1).

Graph 1

Relation of Free Phenolphthalein to Conjugated Phenolphthalein in Urinary Elimination



The curve shows the correspondence between the concentration of free phenolphthalein and of the average concentration of conjugated phenolphthalein.

It must, of course, be understood that every specimen having a concentration, e.g., of 5 mg. per cent of conjugated phenolphthalein does not give a positive test for free phenolphthalein, neither does every specimen having a lower concentration of conjugated phenolphthalein give a negative test for free phenolphthalein. But the general tendency is in this direction (see Graph 1).

Bacteria greatly influence the presence of free phenolphthalein in the urine by causing decomposition of conjugated phenolphthalein to free phenolphthalein on mere standing. Urine containing conjugated phenolphthalein and incubated at 37° C. will show a progressive increase in free phenolphthalein. The same urine kept in the refrigerator or passed through a Seitz filter and then incubated will not show this breaking down of conjugate. Hence prompt testing of the urine is an essential part of the Woldman test.

As previously stated, the data presented mean that "the amount of conjugated phenolphthalein in the urine generally governs the presence or absence

of free phenolphthalein. Hence, any condition which will promote an increased formation or circulation in the blood of conjugated phenolphthalein will lead to the appearance of free phenolphthalein in the urine.'' It is correctly pointed out, as originally stated by us, that in contrast to the patients only 1 per cent of normal persons show free phenolphthalein when the concentration of conjugated phenolphthalein ranged from 1 to 2 mg. per cent, and that only few normal persons show a concentration of conjugated phenolphthalein of above 5 mg. per cent. It is not the 20 to 40 per cent of positive tests in so-called normal individuals which make the Woldman test unreliable, but the 82 per cent of positive results in diseases other than gastrointestinal. *It is this latter fact of over 80 per cent positive Woldman's tests in nonalimentary tract diseases, which renders this test unreliable for diagnosing an ulcerative lesion in the gastrointestinal tract.* Since ailing individuals are the ones who will be subjected to the test, it is important to note how many patients show free phenolphthalein within the range of 1 to 2 mg. concentration of conjugated phenolphthalein and how many of them have a concentration of conjugated phenolphthalein above 5 mg. per cent. In our study with hospital patients, free phenolphthalein was found in 36 per cent of the specimens when their conjugated phenolphthalein concentration was 1 to 2 mg. per cent, and 46 per cent of the specimens showed a conjugated phenolphthalein concentration above 5 mg. per cent. It is thus clear that the excretion of phenolphthalein in normal persons has actually no bearing in the controversy about the usefulness of the Woldman test in ill patients.

INFLUENCE OF DISEASE

Disease has, however, a most marked influence upon the elimination of free phenolphthalein in the urine. Even certain ''mild'' affections of the individual, e.g., cold, sore throat, may greatly change phenolphthalein elimination. As previously stated, in kidney disease there may be practically no phenolphthalein in the urine, while in jaundice very little may be found in the stool and as much as 80 per cent in the urine.

The statement ''All observers recognized early that cardiovascular renal disease and blood dyscrasias gave a high per cent of positives'' can be only partly confirmed by our results. Cardiovascular diseases and blood dyscrasias give a high percentage of positives. Nephritic patients, however, gave in our series the smallest percentage of positives (Table I of preliminary report[1]) since they do have a very small elimination of conjugated phenolphthalein. Rowland's interpretation of the positive Woldman's tests in cardiovascular disease, based on the investigations of Portis and Jaffe,[10] who concluded that: ''When peptic ulcer is the incidental lesion, it is most often associated with cardiovascular disease,'' seems logical and should be further investigated. It, however, does not explain the high incidence of positives in the blood dyscrasias and jaundice.

From the findings in our studies we must disagree with the following statements by Rowland: ''The conjugation of phenolphthalein with sulfates, glycuronates, or other substances is believed to occur in the liver. This function seems to be suspended in jaundice and free phenolphthalein regularly appears in the urine.'' It is our experience that in jaundice the amount of conjugated phenolphthalein in the urine is two to four times that of the normal,

a fact which we believe to be the cause of the regular appearance of free phenolphthalein in the urine. Quantitatively the concentration of conjugated phenolphthalein to total phenolphthalein was found to be about 95 per cent in jaundiced individuals. In other words, in jaundice, free phenolphthalein appears regularly in the urine, not because conjugation of phenolphthalein in the liver is suspended (it may even be questioned whether phenolphthalein is conjugated in the liver), but because the urine contains very large amounts of conjugated phenolphthalein.

It is easy to perceive how false positive tests can be obtained in jaundiced cases, when qualitative examinations are performed. This possibility, however, is excluded when quantitative studies are done, as in these the phenolphthalein is extracted by ether and then determined colorimetrically.

Negative Woldman tests may occur when the solution of phenolphthalein is not absorbed from the stomach or intestine, or when absorption is delayed by other than intragastric or intraintestinal factors, since in those instances even a decrease of conjugated phenolphthalein is found in the urine. Work on the factor of absorption is presently going on in our laboratory and promises to give interesting results.

CONCLUSIONS

1. Additional studies on phenolphthalein elimination support the preliminary observation that the higher the concentration of conjugated phenolphthalein in the urine, the more likely the appearance of free phenolphthalein in it.

2. Phenolphthalein elimination after ingestion of 100 mg. of phenolphthalein in alcoholic solution is much greater within ten hours than the elimination after 300 mg. of powdered phenolphthalein in capsule within twenty-four hours.

3. This difference is obviously due to the factor of absorption.

4. Reports in the literature confirm our observation that Woldman's test[11] is unreliable as a test for gastrointestinal ulcerations.

We wish to express our thanks to Dr. B. Fantus for his valuable suggestions and to Miss E. Redding, R. N., for her technical help.

REFERENCES

1. Steigmann, F., and Dyniewicz, J. M.: Urinary Elimination of Free Phenolphthalein No Test for Gastro-Intestinal Ulceration, Am. J. Digest. Dis. **6**: 120, 1939.
2. Kremer, D. N., Shore, P. D., and Wiesel, B. H.: Phenolphthalein as a Test in Gastro-Intestinal Disease, Am. J. Digest. Dis. **6**: 192, 1939.
3. Suttenfield, F. D.: The Proposed Phenolphthalein Test for Determining the Presence of Gastro-Intestinal Lesions. Results of Ninety-Four Cases, Am. J. Digest. Dis. **6**: 206, 1939.
4. Banks, B. M., and Barron, L. E.: The Phenolphthalein Test in the Diagnosis of Gastro-Intestinal Disease, New England J. Med. **221**: 296, 1939.
5. Notkin, L. J., Kirsch, E., and Saul, A.: Note on the Value of Woldman's Phenolphthalein Test for Gastro-Intestinal Lesions, Am. J. Digest. Dis. **6**: 365, 1939.
6. LeVine, Robert, and Kirsner, J. B.: An Evaluation of the Phenolphthalein Test of Woldman, Am. J. M. Sc. **198**: 389, 1939.
7. Slutzky, B., and Wilhelmj, C. M.: Phenolphthalein as a Test for Gastro-Intestinal Ulceration in the Experimental Animal, Am. J. Digest. Dis. **6**: 449, 1939.
8. Rowland, V. C.: Editorial, Am. J. Digest. Dis. **6**: 396, 1939.
9. Fantus, B., and Dyniewicz, J. M.: Phenolphthalein Studies: Elimination of Phenolphthalein, J. A. M. A. **110**: 796, 1938.
10. Portis, S. A., and Jaffe, R. H.: A Study of Peptic Ulcer Based on Necropsy Records, J. A. M. A. **110**: 6, 1938.
11. Woldman, Edward E.: A Simple Test for Determining the Presence of Gastro-Intestinal Lesions. A Preliminary Report, Am. J. Digest. Dis. **5**: 221, 1938.

THE EFFECT OF SULFANILAMIDE ON ACID-BASE BALANCE*

EVAN W. MCCHESNEY, PH.D., KIMBALL D. SPRAGUE, A.B.,
AND IRVINE H. MARSHALL, M.D., RENSSELAER, N. Y.

SINCE the introduction of sulfanilamide in 1937, many important problems concerning its physiologic effects have been adequately studied. However, one significant aspect of the picture remains in a state of confusion; this concerns the effects of the drug on the acid-base balance of the organism. Although it was rather early recognized[1] that administration of the drug produces a condition simulating acidosis—at least in the sense that there is a decrease in the carbon dioxide combining power of the blood plasma—there has not been a complete agreement among different investigators as to the nature of the disturbance in acid-base balance or its cause.

Observations made on patients[1-5] indicate that administration of sulfanilamide (doses usually 3 to 10 Gm. per day) causes: (1) a decrease in the plasma carbon dioxide combining power, and (2) an increase in urinary carbon dioxide and pH. The same phenomena were observed in dogs by Marshall, Cutting, and Emerson,[6] the doses being from 0.5 to 2 Gm. per kilogram of body weight per day. Thus it is clear that bicarbonates are being lost from the blood and are being excreted in the urine. The general tendency has been to regard the disturbance in acid-base balance due to sulfanilamide therapy as an acidosis, and to supplement such medication with sodium bicarbonate or lactate. Under such conditions cyanosis is ordinarily absent.

The mechanism which brings about the loss of alkali into the urine has remained obscure, but two explanations have been suggested. Marshall, Cutting, and Emerson propose the following: "It is possible that this (alkaline urine) may be due to lack of reabsorption of bicarbonate and base from the glomerular filtrate, the reabsorption of a large amount of the filtered sulfanilamide interfering with that of the bicarbonate." However, they did not present experimental evidence bearing on this point directly. On the other hand, Hartmann, Perley, and Barnett have, on the basis of clinical observations, taken the view that sulfanilamide exerts its influence primarily on the respiratory mechanism, causing hyperpnea with resultant blowing off of carbon dioxide. They assume this to be followed in sequence by: (1) an increase in blood pH, and (2) a compensatory excretion of alkali by the kidneys. They, therefore, feel that the disturbance in acid-base balance should be properly regarded as a "carbon dioxide deficit type of alkalosis." This statement appears to have created some confusion, since they have also raised the question whether routine alkali administration in conjunction with sulfanilamide is "not only not indicated but definitely undesirable under certain circumstances."

*From the Research Laboratories, Winthrop Chemical Company.
Received for publication, April 15, 1940.

In view of the questions raised by Hartmann, Perley, and Barnett, it has seemed desirable to present further evidence bearing on the effect of sulfanilamide on acid-base balance, and, if possible, to demonstrate which of the suggested explanations of the disturbance is correct.

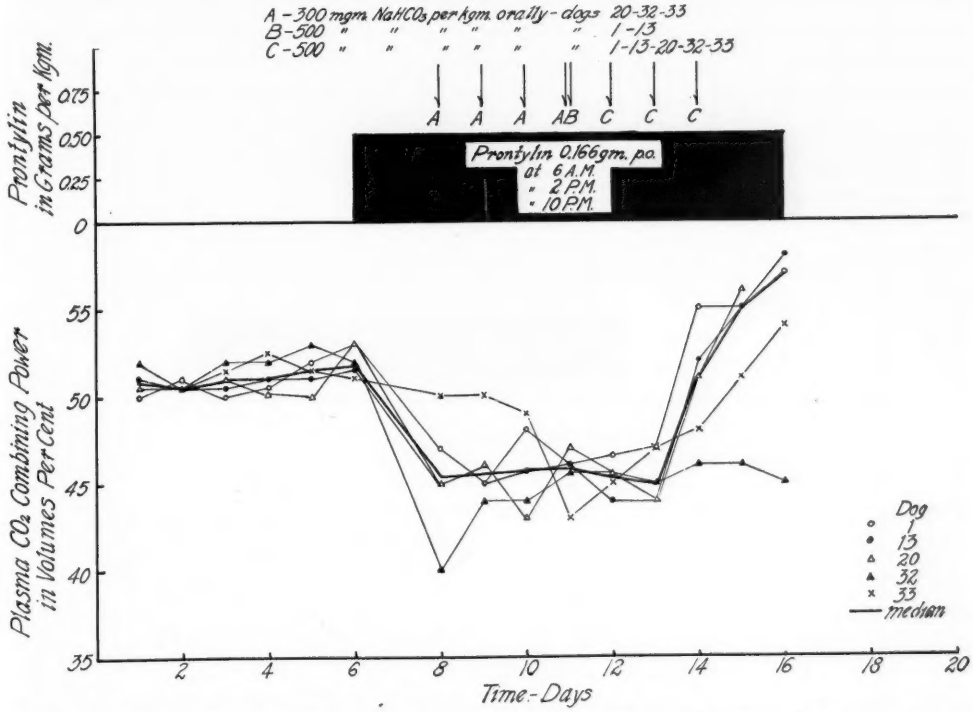


Fig. 1.—Loss of carbon dioxide combining power of plasma caused by sulfanilamide (prontylin) restored by the administration of sodium bicarbonate.

EXPERIMENTAL

I. *Demonstration that sulfanilamide acidosis is relieved by alkali.*—Five adult dogs were used as subjects. During a preliminary control period of five days the carbon dioxide combining power was determined daily until a normal value was established. Medication consisting of 0.5 Gm. of sulfanilamide per kilogram of body weight was then given in a milk suspension by stomach tube (in three equal doses at eight-hour intervals) daily. The carbon dioxide combining power was determined at the same hour each day until it appeared that no further decrease would occur, then the medication was supplemented with the amounts of sodium bicarbonate indicated in the figure (0.3 to 0.5 Gm. per kilogram per day, in three doses) until the original carbon dioxide combining power was restored. The results of this medication sequence are shown in Fig. 1.

Comment. The median normal carbon dioxide combining power for the group of animals was 51 to 52 volumes per cent. The administration of 0.5 Gm. of sulfanilamide per kilogram per day reduced the carbon dioxide combining power to a median value of 45 volumes per cent, after which no further decrease was noted. The administration of 0.5 Gm. of sodium bicarbonate per kilogram per day rapidly restored this value to 52 volumes per cent or more, but dosages

of alkali ranging (in separate experiments) from 0.1 to 0.3 Gm. per kilogram per day were not sufficient to bring about complete recovery.

II. *Demonstration that sulfanilamide acidosis is prevented by the administration of the proper amount of alkali.* A group of five dogs, including four from the previous experiment, was studied in the same manner as before to determine the normal carbon dioxide combining power. When this value was established, the animals were given 0.5 Gm. of sulfanilamide and 0.1 Gm. of magnesium oxide per kilogram of body weight per day as a single dose. The plasma carbon dioxide combining power was determined daily on a blood sample taken just before medication. This treatment was continued for ten days, and the results are shown in Fig. 2.

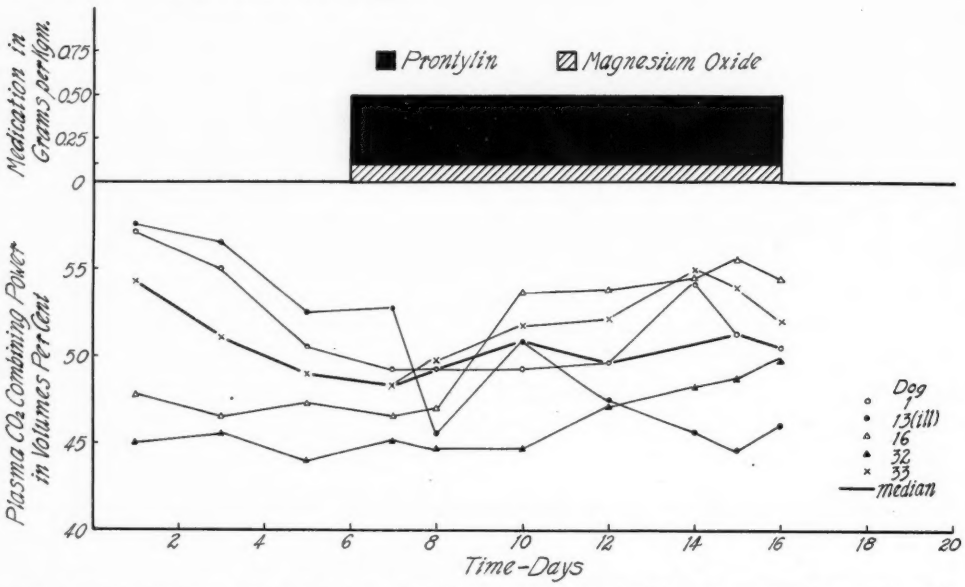


Fig. 2.—Simultaneous administration of sulfanilamide (prontylin) and magnesium oxide. No loss of carbon dioxide combining power results.

Comment. The data represented graphically in Fig. 2 indicate that the normal carbon dioxide combining power for this group of animals is again between 50 and 55 volumes per cent. This value remained practically unchanged during the period of medication, showing that the amounts of alkali and drug given are approximately equivalent physiologically, at least in the dog, and under the conditions of this test.

III. *Demonstration of the order of the changes in the various acid-base factors following sulfanilamide.* A female dog was trained to lie quietly unrestrained on a padded table for long periods of time, was accustomed to retain a urethral catheter and stomach tube, and to permit blood sampling. Respiratory rates and volumes were recorded by means of a pneumograph adjusted to the thorax and connected with a recording tambour, the excursions of which were recorded graphically on a smoked drum. After a satisfactory control period, medication consisting of 0.6 Gm. of sulfanilamide per kilogram of body weight suspended in milk was given by stomach tube (the tube was then removed). Blood samples were taken every thirty minutes and urine samples every fifteen

minutes up to three hours after medication, after which they were taken less frequently. The pH of each sample was immediately taken by means of the glass electrode, the blood determination usually being complete within forty-five seconds after taking the sample, or as soon as a constant reading could be obtained. The urine sample was kept under oil until the blood sample was finished. Respiratory exchange was calculated in arbitrary units; i.e., the rate of

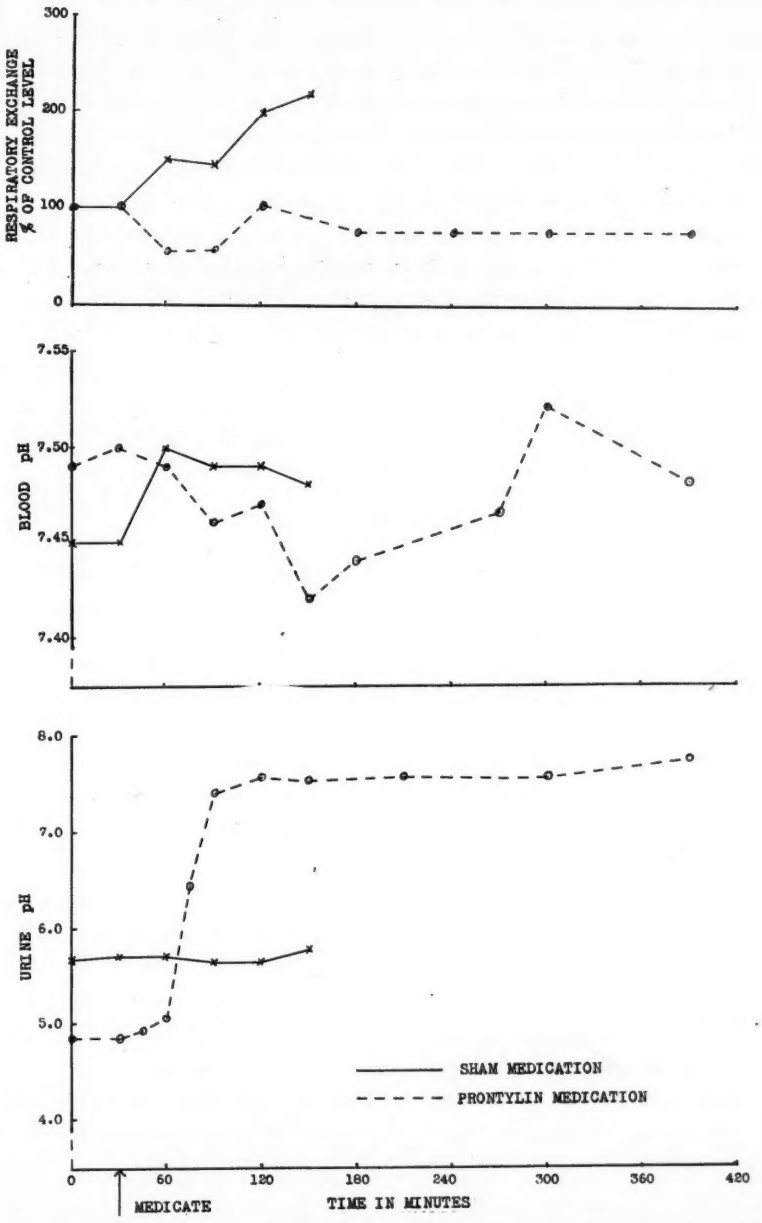


Fig. 3.—Changes in the various acid-base factors following sulfanilamide medication. *A*, Respiratory exchange is given in terms of per cent of the value recorded during the period preceding medication. *B*, Figures given for sulfanilamide medication are medians obtained from three separate experiments. *C*, Figures given for sham medication are averages obtained from two separate experiments.

respiration (which was counted every few minutes) times the recorded amplitude. The respiratory exchange is recorded on the graph in terms of per cent of the value found during the preliminary control period since the absolute value varied from day to day, depending on the temperature, the amount of confusion outside the room, etc. The median observations obtained in a series of such experiments are illustrated in Fig. 3, together with those of control experiments in which a sham medication of an identical volume of milk was given.

Comment. The first effect of medication with sulfanilamide is to depress respiration somewhat. A recovery begins about an hour after medication; the control level is regained and approximately maintained from ninety minutes onward. A definite alkaline trend is first noted in the urine sample taken at forty-five minutes after medication. In this sample the hydrogen-ion concentration has increased twentyfold from the previous sample. Within a hour after medication the urine pH has reached a value about equal to that of the blood, where it remains for the duration of the experiment. The blood pH tends to fall slightly for three hours after medication, after which it rises to a point above the control level, then returns to normal.

In the control experiments the respiratory exchange increased somewhat (more than 100 per cent) as time went on, probably due to nervousness and discomfort, and although the blood pH increased slightly under such conditions, no appreciable change in the pH of the urine was observed.

DISCUSSION

It is shown in these experiments that the disturbance in acid-base balance caused by sulfanilamide may be effectively corrected by supplemental medication with suitable doses of alkalies. Thus the usual decrease in the carbon dioxide combining power of the blood due to sulfanilamide alone can be prevented by giving magnesium oxide simultaneously in a dosage ratio of 100 mg. for each 500 mg. of sulfanilamide. We have also found in other experiments, all details of which are not included here, that 500 mg. of sodium bicarbonate may be substituted for the 100 mg. of magnesium oxide. This ratio of effectiveness (500:100) is practically identical to their ratio of chemical equivalence; namely, 420:100.

The experiment concerning the order of changes in the various acid-base factors following sulfanilamide demonstrates that the explanation of the disturbance suggested by Marshall, Cutting, and Emerson must be essentially correct.* In the first place, alkali begins to appear in the urine in much too short a time for any respiratory mechanism to be involved. We first detected a definite alkaline change in the sample taken forty-five minutes after medication and the urine was, of course, in the process of secretion for some time before this. In the second place, the effect of sulfanilamide on the respiration of the experimental animal was at first depressing, rather than stimulating, as would

*While this paper was being prepared for publication we received a copy of the monograph by Long and Bliss entitled: "The Clinical and Experimental Use of Sulfanilamide, Sulfapyridine, and Allied Compounds" (Macmillan), and we note (page 274) that Beckmann and Bauer, as a result of unpublished experiments, have reached essentially the same conclusions regarding the cause of sulfanilamide acidosis as we have in this paper. Their work was carried out on human beings. The work reported in the present paper was presented by some of us at the Toronto physiological meetings[7] at which time we were naturally unaware of the studies of Beckmann and Bauer.

have been expected if the explanation proposed by Hartmann, Perley, and Barnett were correct. In the third place, we have observed in control experiments hyperpnea and increased blood pH without any change in the pH of the urine. Finally, the blood pH after sulfanilamide tends to *fall* slightly, and to rise later, but only after a respiratory acceleration becomes evident.*

CONCLUSIONS

1. Sulfanilamide causes a disturbance in acid-base balance characterized by loss of alkali from the blood into the urine. This probably has its origin in some type of interference with the reabsorption of bicarbonates from the glomerular filtrate. The phenomenon is, therefore, most accurately designated as a primary alkali deficit due to alkali loss.

2. The alkali deficit which occurs in dogs medicated with sulfanilamide may be compensated by giving alkalies in about the amount of 100 mg. of magnesium oxide or 500 mg. of sodium bicarbonate per 500 mg. of sulfanilamide.

REFERENCES

1. Southworth, H.: Acidosis Associated With the Administration of Para-amino-benzene-sulfonamide (Prontylin), Proc. Soc. Exper. Biol. & Med. **36**: 58, 1937.
2. Hartmann, A. F., Perley, A. M., and Barnett, H. L.: A Study of Some Physiological Effects of Sulfanilamide, J. Clin. Investigation **17**: 465, 1938.
3. Strauss, M. B., and Southworth, H.: Urinary Changes Due to Sulfanilamide Administration, Bull. Johns Hopkins Hosp. **63**: 41, 1938.
4. Brown, A. E., and Bannick, E. G.: The Use of Sulfanilamide and Prontosil Solution, Proc. Staff Meet., Mayo Clin. **12**: 377, 1937.
5. Beckmann, W.: The Effect of Sulfanilamide on Electrolyte Metabolism, J. Clin. Investigation **17**: 537, 1938.
6. Marshall, E. K., Jr., Cutting, W. C., and Emerson, K.: The Toxicity of Sulfanilamide, J. A. M. A. **110**: 252, 1938.
7. McChesney, E. W., Sprague, K. D., and Barlow, O. W.: The Effects of Sulfanilamide on the Acid-Base Balance of Dogs, Am. J. Physiol. **126**: 567, 1939.
8. Hartmann, A. F.: Clinical Studies in Acidosis and Alkalosis: Use and Abuse of Alkali in States of Bicarbonate Deficiency Due to Renal Acidosis and Sulfanilamide Alkalosis, Ann. Int. Med. **13**: 940, 1939.

33 RIVERSIDE AVENUE

*After this paper was submitted for publication it came to our attention that Hartmann[8] has reiterated these same views with new experimental subjects without, however, using any new experimental approach. Our opinion that his views are incorrect remains unchanged. That such is the case is well illustrated by the chart on page 951. Here the urine pH rises immediately after medication, while the serum pH rises slowly (and insignificantly, since within normal limits) over a period of several days. In other words, the "effect" precedes the "cause."

The ammonium chloride medication followed by sulfanilamide seems to have little bearing on the question. It is hardly to be expected that during, or immediately after, an overwhelming acidifying dose (enough to neutralize several times all the bicarbonate in the blood plasma) the kidney would begin to excrete bicarbonates. Any bicarbonate escaping reabsorption at this time would naturally be neutralized in the very acid urine.

THE CAPACITY OF THE KIDNEY TO CONCENTRATE URINE IN ACUTE GLOMERULONEPHRITIS*

RAYMOND GREGORY, PH.D., M.D., GALVESTON, TEXAS

IT IS the purpose of this paper to call attention to the infrequency with which attention is paid to the power of the kidney to concentrate the urine in acute glomerulonephritis and to point out what appears to be an error in most of the discussions of this subject. I will review the literature on the subject and report additional cases of acute glomerulonephritis with impairment of ability to concentrate the urine. My previous experience with a number of cases of acute glomerulonephritis has been at variance with the statements in the usual sources concerning the capacity of the kidney to concentrate urine in acute glomerulonephritis. Unless stated to the contrary, acute glomerulonephritis is used here as a designation for the diffuse variety of this disease.

In the important review of the subject of nephritis by Van Slyke and others,[1] I have found no mention of the capacity of the kidney to concentrate when affected by acute glomerulonephritis. Nor do Peters and Van Slyke[2] discuss this function of the kidney in acute glomerulonephritis. Inasmuch as both papers adequately cover the concentration of the urine by the kidney in chronic glomerulonephritis, one might infer that they do not consider that there is any significant change in this function of the kidney in acute glomerulonephritis.

In this paper the values for the specific gravity of the urine as a criterion of the kidney's concentrating power are based on the work of Addis and Shevky,[3] Lashmet and Newburgh,[4] and Lundsgaard[5] who used a slightly modified Volhard test. Their results vary slightly, but all are in agreement that with proper technique the kidney should concentrate to a specific gravity of at least 1.025. Addis and Shevky found 95 per cent of normal individuals could concentrate above 1.028, and 100 per cent of normal individuals could concentrate above 1.026.

CASE REPORTS

CASE 1.—W. K., a 17-year-old colored school boy, was admitted to the hospital on September 25, 1938. For many months he had prolonged head colds with severe frontal headaches suggestive of sinusitis. The present illness developed insidiously and began with swelling of the face, malaise, and anorexia one month before admission. One week after onset there was swelling of the abdomen, and finally slight swelling of the feet. He was not aware that he had bloody or smoky urine, or any decrease in urine output. He had increasing edema of the face and swelling of the abdomen and progressive weakness.

Examination showed marked edema of face and trunk, and slight edema of the extremities. The edema was of the hard, brawny type and pitted with difficulty. Subsiding bilateral otitis media was present; the right ocular fundus showed papilledema, several

*From the Department of Medicine, Howard University Medical School and the Medical Service, Freedmen's Hospital, Washington, D. C.

Received for publication, May 31, 1940.

small hemorrhages above the disk, arteries of smaller than normal caliber but no exudate. There was marked papilledema on the left. The heart was enlarged to the left, the total transverse diameter being 15 cm., and that of the chest 26 cm. A diastolic murmur was heard along the left border of the sternum and the blood pressure was systolic 158 and diastolic 70. Slight jugular distention, edema of the abdominal wall, slight shifting dullness in the abdomen and slight edema of extremities were present. The spleen descended 2 to 3 cm. below the left costal margin on deep inspiration.

Eight urine examinations were made. All of them showed 2 plus to 4 plus albumin and were acid in reaction. The specific gravity of seven specimens was 1.012, one was 1.015. Only one specimen was positive for blood. Red blood cells were 2,530,000, hemoglobin 34 per cent, white blood cells 13,000, polymorphonuclear cells 88 per cent, lymphocytes 12 per cent. Blood serology was negative.

The patient had fever during his entire stay in the hospital. Both ears drained during most of his hospital course. After one negative culture the blood culture repeatedly showed hemolytic streptococci. The diastolic basal murmur became progressively louder and was associated with a diastolic thrill for the last few days of life. Total blood plasma protein was 7.8 per cent, albumin 3 per cent, and globulins 4.8 per cent. A roentgenogram of the chest showed the total transverse cardiac diameter to be 16 cm., and the chest diameter to be 27.5 cm. On admission the nonprotein nitrogen was 22 mg. per cent, and creatinine 1.7 mg. per cent (frequently found as a normal value in this laboratory). There was a progressive increase in nitrogen retention, which was 85.7 mg. per cent of nonprotein nitrogen, 7.5 mg. per cent of creatinine, and 19 volumes per cent of carbon dioxide combining power the day before death.

The fluid intake was 360 c.c. and 600 c.c. the first and second hospital days, respectively. The output of urine was 300 c.c. and 360 c.c., respectively, the first two days in the hospital. The first twenty-four hours he lost one-half pound, or roughly 250 Gm. During the first week he lost an average of 374 Gm. daily. It is believed that this loss of weight was not all due to fluid loss because of his fever and the extremely small food intake. The specific gravity of the urine was 1.012 on each of the first two days when the output was much diminished. He died on October 18, 1939, twenty-three days after admission.

The clinical diagnoses were acute diffuse glomerulonephritis, acute bacterial endocarditis due to a hemolytic streptococcus on the aortic or pulmonic valves, aortic or pulmonic insufficiency, bilateral otitis media, and hemolytic streptococcic septicemia.

Dr. R. S. Jason, of the Pathology Department, performed a necropsy seven hours after death. The kidneys were enlarged, the combined weights being 705 Gm. The capsules stripped with ease, showing numerous petechial hemorrhages in the cortex. The cut surfaces bulged markedly, the pyramids were dark colored. Microscopic examination of the kidneys showed capsular proliferation, many glomeruli were avascular and showed proliferation of the endothelial cells. Tubular degeneration was marked. An anatomic diagnosis was made of acute thrombo-ulcerative endocarditis of the pulmonic valve; septic emboli to both lungs; diffuse proliferative capsular glomerulonephritis; degeneration of parenchymatous organs and acute splenic tumor; slight dilatation of heart; passive congestion of lungs, liver, spleen, and kidneys; dependent edema; slight ascites and hydropericardium; and bilateral suppurative otitis media. Cultures from the spleen showed hemolytic *B. coli;* from the lung, *B. coli* and hemolytic streptococci.

Comment.—No concentration tests were done as such. I believe it to be important, however, that the specific gravity of the urine was 1.012 on two days when the output was 300 c.c. and 360 c.c., respectively. That diuresis was likely not a factor in the low specific gravities recorded is further shown by the weight loss of this patient. The first day he lost one-half pound, roughly 250 Gm. During the first week he lost five and one-half pounds, an average of approximately 374 Gm. per day. This was probably not all due to fluid loss because he ate very little, in spite of the fever. On the days when the specific

gravity was 1.012 and the urinary output was very low, the urine contained three-plus albumin. The specific gravity corrected for the albumin would likely have been 1.011 or 1.010.

CASE 2.—H. A., a 51-year-old colored male janitor, was admitted to the hospital on March 29, 1939, and was discharged improved on June 14, 1939. His complaints were shortness of breath, swelling of the feet, legs, and abdomen, and weakness. In February, 1939, he had a disease characterized by fever, generalized aches and pains, and diagnosed as "grippe" by a physician. About February 20 he returned to work but became progressively weaker. Shortness of breath and swelling of his abdomen gradually developed. Swelling of the feet and of the face occurred a few days before admission.

Examination showed him to be pale. He could lie flat without respiratory embarrassment. His heart was of normal size, his mechanism was normal, and his blood pressure was systolic 120 and diastolic 86. Edema of the abdominal wall, especially of the flanks, and two-plus pitting edema of the legs were noted. The fundi oculi were normal.

The urine was cloudy, acid, had a specific gravity of 1.014, and contained three-plus albumin. It was otherwise normal. The blood had red cells 4,570,000, hemoglobin 72 per cent, white cells 13,350, normal differential. Hinton and Eagle precipitation tests were negative.

During the patient's entire stay in the hospital he had no fever and a steady though slow improvement. Weight nineteen days after admission was 189 pounds; two days before discharge, 163 pounds. The weight on admission was probably greater than 189. More than twenty blood pressure recordings were systolic 110 to 120 and diastolic 70 to 80. On March 30, 1939, the venous pressure was 8 cm. of blood. On April 4, 1939, 1,300 c.c. of chyliform fluid was withdrawn from the abdomen, the specific gravity of which was 1.010. On April 12, 1939, the total blood plasma proteins were 4.7 Gm., albumin 2.3, and globulin 2.4. In spite of a four-plus albuminuria the maximal concentration of the urine was 1.018 sixteen hours after abstaining from food and drink. A urea clearance of 9 per cent of maximal clearance and a blood nonprotein nitrogen of 91 mg. per cent were found. On May 6, 1939, the red blood cell count was 2,850,000 and hemoglobin was 40 per cent. On May 12, 1939, the red blood cell count was 3,050,000. On May 18, 1939, urea clearance was 13 per cent of maximal normal; total proteins 4.9, albumin 1.5, globulin 3.4, and the blood nonprotein nitrogen 53.2 mg. per cent. On May 25, 1939, the twenty-four-hour specimen of urine measured 1,100 c.c. and contained 17 Gm. of protein. On June 5, 1939, the nonprotein nitrogen was 31.6 mg. per cent, creatinine 3.0 mg. per cent (normal values in this laboratory often 2). On June 9, 1939, total protein was 6.0 Gm., albumin 3.5, globulin 2.5, urea clearance 15 per cent of the standard clearance.

Clinical diagnoses: Acute glomerulonephritis, ascites, and uremia.

Comment.—Twelve urine specimens contained three-plus to four-plus albumin for the first six weeks in the hospital. Red blood cells were found in the urine only infrequently. Of fifteen urine examinations twelve had a specific gravity of 1.017 or below (1.013 to 1.017); two were 1.018; one was 1.020 one month after admission. In spite of marked edema, ascites, and albuminuria on admission, the specific gravity of the urine was 1.014. On April 20, 1939, in spite of a four-plus albuminuria, the maximal concentration of the urine was 1.018 sixteen to seventeen hours after no food or fluids. It appears from the above data that the impairment of the tubular function was not as extreme as that of the glomerular function. But if the correction is made for the albumin content of the urine, the specific gravity of the urine indicates a greater degree of tubular damage. It is known[6] that urines which contain a large amount of protein have more than 0.5 per cent. One quantitative estimation of this patient's urine indicated that he had 1.5 per cent. It seems fair,

therefore, to assume that the urines which had three- to four-plus albumin contained approximately 1 per cent albumin. A correction[7, 8] for the amount of albumin would result in a maximal concentration of 1.015 instead of 1.018.

CASE 3.—O. T., a colored school girl, 15 years of age, was admitted to the hospital on December 6, 1938, with the complaint that four weeks before she was admitted her illness had begun with severe frontal headaches and drowsiness associated with swelling of the face, particularly the eyelids, and feet. The edema subsided during the day and recurred during the night. Seven days before admission visual impairment became so marked that she consulted an ophthalmologist. She gave no history of a previous respiratory or any other type of infection for many months prior to onset of the illness. The urine assumed a darker color during the present illness, but she had not been aware of bloody or smoky urine or any diminished volume. Urinary frequency was four times daily and once at night. She said her face was less swollen than at the beginning of her illness.

Examination showed the face and lids edematous. The blood pressure was systolic 115 and diastolic 80, the heart and lungs were normal, the tonsillar lymph nodes were enlarged, the tonsils were absent, the spleen was not felt, the abdomen was normal. The patient was in a good nutritional state and reflexes were normal. Fundus examination showed marked bilateral papilledema, numerous splinter hemorrhages in both eyes, veins engorged, very slight unevenness of caliber of arteries, a few cotton-wool exudates close to the disks, macular areas normal, and two flame-shaped hemorrhages a disk's diameter above right disk.

The urine was acid and cloudy and had a specific gravity of 1.020; it gave a three-plus albumin reaction, and was negative for sugar, acetone, and bile. The sediment showed many long granular and cellular casts, numerous red blood cells, and the benzidine test on the sediment was strongly positive for blood. During the next two months fifteen examinations of the urine were made. A three-plus albuminuria was present on all but two occasions, when it was two plus. Microscopic and/or chemical tests for blood were positive in all but three specimens. Granular casts were found seven times in the fifteen examinations.

The blood on December 8, 1938, showed red blood cells 3.48 million, hemoglobin 54 per cent, white blood cells 4,050, essentially normal differential. On January 3, 1939, red blood cells showed 2.4 million, hemoglobin 28 per cent; the reticulocyte count on January 16 was 4.2 per cent. The red blood cell counts remained from two to three million and the hemoglobin from 28 to 52 per cent. The white blood cell count was low for the first six weeks in the hospital when it went to 11,400. There was a constant leucocytosis from 18,450 on January 20 to 27,800 on January 23 and 22,550 on February 1. On and after February 20 the polymorphonuclear cells were 83 per cent or greater. On December 7, 1938, one day after admission, nonprotein nitrogen was 54.5 mg. per cent, creatinine was 3.0 mg. per cent, carbon dioxide combining power was 53 volumes per cent; on December 12 nonprotein nitrogen was 46 mg. per cent, creatinine was 1.9 mg. per cent; on December 19 nonprotein nitrogen was 28 mg. per cent, and creatinine was 1.4 mg. per cent.

The patient had fever during her entire stay in the hospital; on several occasions her temperature was 104° and 105° F. On December 11, 1938, she was clinically improved, the edema was rapidly disappearing, and she was mentally more alert; the following day her blood pressure was systolic 114 and diastolic 78. Physical examination revealed a productive cough but normal lungs. On December 15, 1938, papilledema decreased, only one flame-shaped hemorrhage remained in the right eye; the exudates previously seen had disappeared. Three days later eye grounds were essentially the same. She was given a transfusion of 400 c.c. of citrated blood on January 4, 1939. Two days later the specific gravity of her urine was 1.012; on January 8 the specific gravity of the urine was 1.012.

There were signs of fluid in left chest on January 21, 1939; pericardial friction rub was heard on January 23. The patient was having pain over the precordium, and both the right and left sides of her heart were considered enlarged. Tubular breathing was heard over the left base posteriorly. The question whether this was due to pericardial effusion or pleural or pneumonic disease was raised, but no conclusive decision was reached.

On January 30 two hemorrhages and exudate and papilledema were seen in the left fundus; on January 31 the Rumpel-Leede capillary fragility test was positive. The blood pressure was systolic 140 and diastolic 98. Nonprotein nitrogen was 160 mg. per cent, creatinine was 3.0 mg. per cent, total blood plasma proteins were 6.25, albumin was 2.10, and globulin was 4.15; uremic frost was observed along hair margins. On February 1 total protein was 6.25 Gm. per cent, albumin 2.10 Gm. per cent, globulin 4.15 Gm. per cent, nonprotein nitrogen 160 mg. per cent, creatinine 3.0 mg. per cent; the following day nonprotein nitrogen was 210 mg. per cent and creatinine 3.5 mg. per cent.

Concentration Tests.—(All water and food were withheld fourteen to sixteen hours and the bladder was emptied one to two times before the specimen was obtained for specific gravity estimation.) On December 7, 1938 (the day after admission, four weeks after onset), the specific gravity was 1.019, albumin was three plus; on December 15, specific gravity 1.018, albumin three plus; on December 23, specific gravity 1.017, albumin three plus; January 5, 1939, specific gravity 1.010, albumin two plus; January 8, specific gravity 1.016; January 9, specific gravity 1.012, 90 c.c.; January 10, specific gravity 1.006, 90 c.c.; January 12, specific gravity 1.012, 120 c.c.; January 13, specific gravity 1.012, 150 c.c.; January 14, specific gravity 1.010, 210 c.c.; January 15, specific gravity 1.010, 150 c.c.; January 16, specific gravity 1.007, 180 c.c.

The course grew progressively worse and the patient died February 4, 1939, on the sixty-second hospital day. Permission for necropsy was not obtained.

Clinical diagnoses: Acute glomerulonephritis terminating in uremia, septicemia of undetermined etiology, uremic pericarditis with effusion.

Comment.—This patient probably had impairment of concentrating capacity when she was admitted to the hospital dehydrated, edematous, and with a urine specific gravity of 1.020 and a three-plus albuminuria. There was a gradual decrease in this function until the concentrating capacity was fixed at 1.010 to 1.012 after January 9, 1939. The necessity for making corrections for the albumin content of the urine in specific gravity estimations in concentration tests is again emphasized.

CASE 4.—J. R., a 29-year-old colored clerk, was admitted to the hospital on October 10, 1938, complaining of shortness of breath and a bad cough. The present illness began three weeks before admission with a "head and chest cold" after he had become thoroughly soaked in a rainstorm. He had chilly sensations and fever from the onset of his illness. Several days before admission he developed extreme shortness of breath, edema of lower extremities, headaches, and dizzy spells. Urinary frequency was five to six times in the day and three to four times during the night. Two months before admission he had had gonorrhea which had been treated successfully by a physician.

He exhibited a great amount of unproductive coughing. Examination showed orthopnea. The conjunctivae were markedly blood shot; face and eyelids were "puffy." The fundi oculi showed no pathology. The jugular veins were slightly distended and there were congestive râles in the lung bases. The heart was enlarged to the left anterior axillary line in the fifth interspace. The apical thrust was not forceful. There was a gallop rhythm and pulsus alternans, the heart rate was 140, and the blood pressure was systolic 196 and diastolic 160. There was a small amount of fluid in the abdomen, the liver was tender and extended 4 cm. below the right costal margin. There was a grade two to three edema of both lower extremities up to the knees and slight tenderness in the right costovertebral angle.

The urine on admission showed a specific gravity of 1.026 and contained four-plus albuminuria. It was cloudy and contained numerous red blood cells per low-power field and occasional granular casts. The blood showed red blood cells 4.2 million, hemoglobin 85 per cent, white blood cells 8,300, neutrophiles 80 per cent, lymphocytes 19 per cent, and basophiles 1 per cent.

He was given absolute bed rest, digitalis, and sedatives; fluid and salt were restricted. His temperature was within normal limits with the exception of two dif-

ferent days when it reached 100.4° F. rectally. Blood pressure was systolic 196 and diastolic 160 on admission and remained elevated during his stay in the hospital. The weight on admission was 158 pounds; one week later, October 17, 144 pounds; October 24, 139; October 31, 134; November 7, 131; November 14, 129. On October 11 nonprotein nitrogen of the blood was 100 mg. per cent, creatinine 3.0 mg. per cent. On October 15 nonprotein nitrogen was 50 mg. per cent, creatinine 2.3 mg. per cent, carbon dioxide combining power 53 volumes per cent; October 24, nonprotein nitrogen 40 mg. per cent, creatinine 1.3 mg. per cent.

The urine was acid on October 11, had a specific gravity of 1.022, two-plus albumin, six red blood cells per high-power field, and an occasional granular cast. On October 14 the urine was pale yellow, acid, had a specific gravity of 1.009, and three-plus albumin, white blood cells 3 to 4, no red blood cells, one to two granular and hyalin casts; October 15, urine pale, neutral, specific gravity 1.010, two-plus albumin. He developed signs of a pulmonary infarct in the right lower lobe. On October 22 the signs of congestive failure and pulsus alternans had disappeared; urine acid, specific gravity 1.018, two-plus albumin; October 28, urine acid, specific gravity 1.010, albumin one plus, few red blood cells, and three granular casts per high-power field; October 31, urine yellow, acid, specific gravity 1.017. Hinton and Eagle precipitation tests were negative. He was discharged on November 14, 1938, as improved.

Clinical diagnoses: Acute glomerulonephritis, cardiac dilatation, congestive heart failure, and pulmonary infarct.

Comment.—This man unquestionably had congestive heart failure. The clinical picture otherwise was characteristic of acute glomerulonephritis. I believe that the nonprotein nitrogen value of 100 mg. per cent was too high to be explained on the basis of congestive heart failure, although this probably contributed to the renal failure. The number of red blood cells was also much higher than the few one commonly sees in the urine due to passive congestion of the kidneys. I have concluded that the primary disease was acute glomerulonephritis and that the heart failure was that which is not uncommonly associated with acute glomerulonephritis.

My justification for including this case in this paper is that the urinary specific gravity on admission was 1.026 with a four-plus albuminuria and marked congestive heart failure, both of which factors raise the specific gravity of the urine. It should be noted also that the specific gravity of the urine for the first week after the first specimens gradually reached the level of 1.010. It may be argued that this was in part due to diuresis.

My principal purpose in including this case in this report is to emphasize that there may be nitrogen retention in acute glomerulonephritis with less decrease in the tubular function of concentration than one usually sees in chronic glomerulonephritis.

REVIEW OF LITERATURE

It is stated in commonly consulted sources[9-19] that the specific gravity of the urine in acute glomerulonephritis is normal or even high. Some of the statements, however, are conflicting. Boyd,[12, 19] for example, says that acute nephritis is characterized by a urine of high specific gravity and a low urea content. It appears to me that these two statements are incompatible in view of the fact that urea is one of the substances which is normally concentrated to a greater degree than any other substance in the urine. Unless glomerulonephritis is associated with a decreased formation of urea, or there is a unique interference with the capacity of the kidney to concentrate urea alone, neither

of which is true so far as I am aware, it would seem that it is impossible for a high specific gravity of urine to exist with a low urea content. The statement relative to the low urea content of the urine implies either specific impairment of the glomerular filtration of urea, increased tubular absorption of urea, or some impairment of the tubular function of concentration in general. The latter seems a more logical explanation of a low urine content of urea.

The statements in Best and Taylor[13] also are ambiguous. Referring to the pathology of acute glomerulonephritis they say, "Such a severe reaction must of course, if widespread, cause a high degree of renal insufficiency. The following are among the chief features which may appear: retention in the blood of non-protein nitrogenous products; edema; . . . . . ; and the passage of a small quantity of urine with a high specific gravity." These authors[14] then proceed to define renal insufficiency as a "reduced capacity of the kidney to carry out its functions;" and include a diminished concentrating power as an invariable accompaniment of renal insufficiency.

Bell[20] also appears ambiguous or inconsistent in his discussion of the concentrating capacity of the kidney in acute glomerulonephritis. For example, on page 497 he states, "In this publication clinical acute nephritis includes all cases of acute renal disease that exhibit a definite impairment of renal function indicated by retention of nitrogenous products, decreased ability to excrete P.S.P., *inability to form a concentrated urine,* loss of large amounts of protein in the urine, bleeding from the parenchyma of the kidney and severe oliguria and anuria." While on page 523 he says, "The total glomerular filtrate is decreased in amount in severe cases and it is distributed through more tubules than under normal conditions, in which only about one-third of the nephrons are active at any one time. The decrease of glomerular filtrate tends to cause retention of metabolites. The distribution of filtrate through a larger number of tubules where it is exposed to a greater reabsorption tends to produce a concentrated urine."

I offer the following criticism of this concept. In the first place, Bell includes in his definition of acute nephritis an inability to form a concentrated urine. He then proceeds to show why the severe forms of acute glomerulonephritis are able to concentrate their urine. In the second place, this concept appears to be based on two assumptions which may or may not be true. At any rate I know of no proof that they are true. Bell appears to assume that all the glomeruli in acute nephritis are open all the time and never shut down. If this really were the case, it would appear logical to believe that the constant filtration through the glomeruli would not allow the urine to remain in the tubules sufficiently long to concentrate it, inasmuch as it seems likely that the greatest amount of concentration may occur in the tubules when the glomerular capillaries are not open. This concept also fails to take into consideration that all glomeruli and all capillaries of each glomerular tuft are not normally open at any one time. This concept of Bell's further assumes that the tubules associated with these glomeruli are functioning normally, in spite of his statement that in severe cases the glomeruli are practically bloodless from intracapillary and extracapillary exudation and proliferation.

If the latter is so, and it is a common teaching of pathologists, it appears to me that it is not in accord with the facts to assume that the tubules of these nearly bloodless glomeruli can function normally when they too are practically deprived of their blood supply by the glomerular pathology. This statement is based on the work of Hou-Jensen[21] and Clara[22] who have shown that at least 97 per cent of all the arterioles of the medulla (tubules) of the kidney arise from the vasa efferentia.

On the basis of the work of Alving and Van Slyke[23] who showed that concentrating capacity may return many months after return of urea clearance, it seems logical to me to believe that the disturbed tubular function in glomerulonephritis may be due not only to a diminished blood supply from bloodless glomeruli, but also to a specific effect of the toxic agent on the capillaries of the tubule itself.

As a further example of the widespread and firm belief that the kidney in acute nephritis retains its capacity to concentrate the urine well, Albright[24] states, in the discussion of a case, that the differential diagnosis rests between acute and chronic nephritis; but that decision against acute nephritis was made because, among other things, the patient failed to concentrate the urine above 1.014 maximum. The case was shown at autopsy to be one of acute glomerulonephritis.

In contradistinction to the above case, which showed a nitrogen retention and a low capacity to concentrate, may be mentioned another case from the same source[25] (Case 12, Table I). The onset of illness was three weeks before admission, with marked edema of the extremities, genitals, and face; blood pressure was systolic 150 and diastolic 110; albumin and casts were present in the urine; nonprotein nitrogen was 38 mg. per cent, and dilution and concentration test was 1.002 to 1.032. The tubular function was normal and there was no gross evidence of glomerular failure as observed by the normal blood nitrogen. In other words, it appears that if the capillaries of the glomeruli are sufficiently open to prevent nitrogen retention, the tubules get enough blood to allow them to concentrate the urine normally. And if the glomerular capillaries are sufficiently damaged to cause nitrogen retention, the tubules are sufficiently disturbed functionally to result in impaired concentration.

In spite of Christian and O'Hare's[15] statement regarding the high specific gravity of the urine in acute nephritis, their reported cases would appear to be out of accord with their statement. The interpretation, however, is difficult in view of the fact that we do not know the method by which the urine was collected. For example, Case 1 had fourteen specimens examined over a three-month period. Only three of these are above specific gravity 1.020, and ten specimens range from specific gravity 1.013 to 1.019. One specimen was 519 c.c. for twenty-four hours and had a specific gravity of 1.015. Case 3 had nineteen urine specimens examined over a period of fifty-two days, with a specific gravity ranging from 1.010 to 1.017. Most of these were twenty-four-hour specimens. This patient was followed for six months after this, the specific gravity varying between 1.011 and 1.022. Eighteen months after onset of illness the specific gravity was 1.025. Case 5 had twelve specimens examined, with the specific gravity ranging from

TABLE I

SUMMARY OF TWENTY-TWO CASES OF ACUTE GLOMERULONEPHRITIS FROM THE LITERATURE AND FOUR ADDITIONAL CASES WHICH SHOW IMPAIRED CAPACITY TO CONCENTRATE THE URINE

CASE NO.	AGE SEX	PRECEDING INFECTION	INTERVAL AFTER ONSET OF OBSERVATION	BLOOD PRESSURE	URINE	CONCENTRATION TEST—MAXIMUM	UREA CLEARANCE	BLOOD NITROGEN	EDEMA	OLIGURIA	AUTHOR
1	45 Male	Cellulitis of hand, lymphangitis of arm	At once	185/115	Blood, W.B.C., albumin, casts	1.020 (Addis)	Not done	Blood urea 98 mg. %	Marked	Positive	Addis and Oliver
2	24 Male	Scarlet fever	3 weeks after onset of symptoms	180/105	R.B.C., W.B.C., albumin, casts	1.018 (Addis)	Not done	Blood urea 189 mg. %	Marked	Positive	Addis and Oliver
3	29 Male	Strept. hemolyticus septicemia	3 weeks. Dead 4 weeks after onset	118/50	R.B.C., W.B.C., albumin, casts	1.014 (Addis)	Not done	Blood urea 206 mg. %	Positive		Addis and Oliver
4			6 weeks after onset	170/55 110/65	Blood, albumin	1.001 to 1.010 1.001 to 1.024 (Volhard)	39 c.c./min., 52% maximal normal	Blood urea 160 mg. %			Rehberg
5	31 Male	Cut finger, axillary abscess	3 weeks 6 weeks	150/100	Gran. and hyalin and cellular casts, albumin, 2 plus	Onset 3/12; 4/2: 1.012 to 1.020; 4/25: 1.017	Not done	Not mentioned	Marked		Longcope
6	26 Male	"Cold"	7 weeks	164/92	4 plus albumin	1.012 to 1.020	Not done	Not mentioned	Positive, feet		Longcope
7	48 Male	Not given	At onset	160/90	Hematuria	1.012 max.; two observations at 3 mo. interval 1.020	12% avg. normal; 80% after 3 mo.	Not given	2 plus		Alving and Van Slyke

8	43 Male	Not given	1 month	158/90	Hematuria	1.011; still impaired to 1.012 after 3 mo.	16% avg. normal; normal after 3 mo.	Not given	Absent on admission		Alving and Van Slyke
9	11 Male	Not given	13 months		Hematuria	After 5 yr.; max. 1.018	Normal clearance for 5 yr.	Not given			Alving and Van Slyke
10	Male	Boil 3 weeks before admission	Uncertain	140/95	Blood, many R.B.C., W.B.C., albumin, casts	1.006 to 1.014	Not done. No P.S.P. in 1 hr.	N.P.N. 101 mg. %	Positive		Case reports, Mass. General Hospital
11			2 weeks before admission	150/110	R.B.C., W.B.C., albumin, casts	1.002 to 1.032	Not done	N.P.N. 38 mg. %	Marked extremities, puffiness over eyelids and face		Case reports, Mass. General Hospital
12	17 Male	Sore throat	8 days	Not given	Albumin, hematuria, marked	1.013 max.; 1.015 after 3 mo.	Creatinine clearance 14 c.c./min.; clearance had returned to 80 c.c. after 3 mo.	Blood urea 90 mg. %	Not given		Holten
13	19 Female	Erysipelas onset 1/17	3 weeks	Not given	Albumin, hematuria	2/9: 1.017; 2/16: 1.010; normal 6 mo.	Creatinine clearance filtration 9.5 c.c./min.; low 17 mo.	Blood urea 2/9: 140 mg. %; 2/16: 200 mg. %	Not given		Holten
14	5 Male	Scarlet fever	Immediately after onset			1.010 on admission; normal after 41 days	Slightly impaired	Blood urea 90 mg. %			Holten

TABLE I—CONT'D

CASE NO.	AGE SEX	PRECEDING INFECTION	INTERVAL AFTER ONSET OF OBSERVATION	BLOOD PRESSURE	URINE	CONCENTRATION TEST—MAXIMUM	UREA CLEARANCE	BLOOD NITROGEN	EDEMA	OLIGURIA	AUTHOR
15	27 Female	Sore throat	Immediately after onset			1.012 maximal normal 3 mo.	Creatinine 65 c.c./min. Normal, this person 115 c.c.; normal 4 mo.	Blood urea 50 mg. %			Holten
16	18	Sore throat	10 days	Normal throughout		1.010 to 1.016 13 days after onset; 1.009 to 1.017 20 days after; 1.000 to 1.023 40 days after onset	Not done	N.P.N. 52 mg. % 13 days after onset; 46 mg. % 20 days after onset	None		Donath
17		Sore throat	14 days	Normal	Blood, albumin, casts	1/14: 1.014 to 1.018; 1/21: 1.012 to 1.020; 1/26: 1.010 to 1.025; 2/1: 1.003 to 1.024	Not done	1/14: N.P.N. 62 mg. %; 1/21: N.P.N. 38 mg. %			Donath
18	26	Sore throat 7/11/29	8 days	Normal	R.B.C., pus cells, albumin, casts	7/19: 1.010 to 1.016; 8/6: 1.012 to 1.023		N.P.N. 36 mg. %	None		Donath
19	Adult			160	Blood, albumin 4 %	1.015 maximum					Volhard and Fahr
20	Adult			144	Blood, albumin 0.25 %	1.018					Volhard and Fahr

21	Adult					1.013 maximum		N.P.N. 80 mg. %		Yes 350 c.c./24 hr.	Volhard
22	27 Male			Normal	Marked hematuria	1.010 fixed				Yes 200 c.c./14 hr.	Volhard
W.K.	17 Negro Male	Frequent sore throats, sinusitis, otitis media	1 month	158/70	Hematuria, albuminuria	1.010 to 1.012	Not done	N.P.N. 22 mg. % on admission	Face, trunk	Yes 300 c.c.; 360 c.c. 2 days	Gregory
H.A.	51 Negro Male	"Grippe"	2 to 4 weeks	110-120 70-80	Slight hematuria, albumin, casts	1.018; 4 plus albuminuria	9% of maximal normal	N.P.N. 91 mg. %	Face, trunk, ascites	At onset	Gregory
O.T.	15 Negro Female	None known	4 weeks	Always normal 115/80	Marked hematuria, albumin, casts	Decreased from 1.017 on Dec. 23 to 1.010 and 1.012 on Jan. 9	Not done	N.P.N. 54 mg. % on admission; 12/19: 28 mg. %; 2/2: 210 mg. %	Face, eyelids	Not noted	Gregory
J.R.	29 Negro Male	"Head and chest cold"	Less than 3 weeks	196/160 on admission; 140/100 when discharged	Hematuria, albumin, casts	1.004 to 1.015, 3/27/39	33% maximal normal on May 8	N.P.N. 10/10: 100 mg. % on admission; 10/15: N.P.N. 50 mg. %; 10/24: N.P.N. 40 mg. %	Face, trunk, extremities, ascites	Present on admission	Gregory

1.008 to 1.014. Eleven months after onset of illness, the specific gravity was 1.019; forty-two months after, it was 1.028. The last two specimens were single-voided specimens. In the absence of specific knowledge of the method of collecting the specimens, one cannot state too definitely that the figures are evidence of a diminished concentration capacity of the kidneys. It may be fairly assumed that there was some uniformity of collection of specimens, and the trend upward in specific gravity, with the passage of time, may possibly be interpreted as evidence of previous impairment of concentration.

Difficulty also arises in connection with Christian and O'Hare's cases in the matter of deciding to what extent diuresis may have been a factor in the low specific gravities reported. The fact, however, that Case 1 had urinary specific gravities of 1.012, 1.014, and 1.015, while at the lowest recorded weight of 58.2 kg., would indicate that diuresis was probably not the cause of the low specific gravity. In Case 3, the specific gravities of 1.010 and 1.014 were also on days when the lowest weight of 51.2 kg. was recorded. This fact appears to render diuresis an unlikely explanation for the low specific gravity. Diuresis was probably not the explanation of the low concentrations of the urine in Case 5, as evidenced by specific gravity of 1.008 and 1.011, with the highest weight of 62.8 kg. on admission, which fell in eighteen days to 52.8 kg., the lowest recorded; the urine, however, continued to show specific gravities of 1.009, 1.010, and 1.011 at the lowest weight recorded.

The report of Murphy, Grill, and Moxon[26] is also difficult to interpret accurately regarding the concentrating capacity of the kidney in acute glomerulonephritis. It is of suggestive importance, however, that in twenty patients who became chronic cases the second specific gravity recorded in each case (one to four months after the first) was lower than that on the first study. On the other hand, the specific gravity in ten patients who recovered, the second observation being one to four months after the first, the specific gravity at the second examination, with one exception, was higher than that of the first examination. It seems fair to assume some uniformity in the manner in which the first and second urines were collected in each instance.

I have been able to find but few instances in which the concentrating capacity of the kidney in acute glomerulonephritis has been studied by any of the concentration tests. Most of the reports on acute glomerulonephritis, therefore, are of little value to the purposes of this article. Yet in one year's time I have observed four cases of acute glomerulonephritis with impairment of concentration capacity on a relatively small medical service.

While the general sources cited indicate that the capacity of the kidney to concentrate is unimpaired in acute nephritis, most of the special works on the kidney do not agree with this viewpoint. I have found twenty-one cases of acute nephritis with such impairment found by ten different observers in the relatively recent literature. These cases are summarized in Table I, together with the four additional cases described here.

Addis and Oliver[27] reported three cases of acute glomerulonephritis with unquestioned impairment of concentration (Cases 1, 2, 3, Table I). Rehberg[28] studied six cases of acute glomerulonephritis from which he concluded that the tubular function may also be affected in this stage of the disease. He gives data

from only one case, however (Case 4, Table I). Longcope[29] studied nine cases of acute glomerulonephritis, but only two of these had studies directed at the power of the kidney to concentrate the urine; both of these showed impaired concentration (Cases 5 and 6). The Addis technique was observed in one of these and the volume of the twelve-hour night urine was found normal. The low specific gravity, therefore, was not due to diuresis.

Volhard and Fahr[30] state that the concentrating capacity is impaired in acute glomerulonephritis as follows: "The lower the specific gravity of the urine, with small amounts of urine, the greater the kidney damage"; and "In general, in the more severe forms of acute glomerulonephritis the rule is the concentrating capacity of the kidney suffers; and indeed the more so, the more intensively and extensively the glomeruli are diseased." They give very brief summaries of two cases which demonstrated this feature (Cases 19 and 20).

Although Volhard states in another place[31] that the concentrating capacity of the kidney was only slightly or not at all disturbed in acute glomerulonephritis, the two cases which he cites had markedly impaired capacity to concentrate the urine (Cases 21 and 22).

Donath[32] has reported three patients with acute glomerulonephritis who showed impaired concentrating capacity of the kidney (Cases 16, 17, 18). While in two patients there was very slight nitrogen retention and in one there was none, all three had impaired concentration at times when the blood nitrogen was normal. There was also impairment of the capacity to dilute the urine. Both types of impairment persisted after red blood cells, casts, and albumin disappeared.

Holten[33] studied 25 patients with acute glomerulonephritis with the Volhard-Strauss concentration technique and the creatinine clearance test of Holten and Rehberg.[34] In this group there were four instances (Cases 12 to 15) of either moderate or severe impairment of concentration, and two other instances of slight impairment. In all instances in which elevation of blood nonprotein nitrogen occurred, there was diminished capacity to form a concentrated urine. In one patient the filtration rate returned to normal before the concentration test. This was also shown by one of the patients of Alving and Van Slyke. The possible significance of this will be discussed in connection with the work of Alving and Van Slyke.

Holten[33] is well aware, of course, that the concentrating capacity of the kidney may be retained in acute glomerulonephritis, but states further that he has never seen it retained in patients with serious impairment of the filtration rate (below 10 c.c. per minute). He cites Volhard's explanation for the maintenance of the concentrating capacity of the kidney in acute nephritis, namely, that the tubules are able to excrete solids to a normal extent and the glomeruli are unable to excrete water.

Alving and Van Slyke[23] have shown results which are interesting and pertinent to the present paper in their comparative values of the urea clearance and concentration tests in Bright's disease. In three transitory cases of glomerulonephritis (hematuria, proteinuria, and initial edema left no doubt that each patient had an acute nephritis) both urea clearance tests and concentration tests

were normal throughout. They also had three cases (Cases 7, 8, 9) of acute glomerulonephritis which had impairment of both the urea clearance tests and concentration tests. Their fourth case had an original urea clearance of 12 per cent of the average normal and a maximal concentrating capacity of 1.012. Three months later the clearance test had risen to 80 per cent of average normal, but the concentration capacity by both the Addis-Shevky and Lashmet-Newburgh tests had risen only to from 1.019 to 1.020. Later this also returned to normal. Their fifth case also showed a marked lag in the return to normal of concentration capacity after the clearance had returned to normal. Three months after onset the urea clearance was normal, but the Addis test showed a maximal concentration of 1.012 which later returned to normal. Their seventh case did not have a return to the normal concentration capacity until five years after the urea clearance was normal.

Lyttle[35] calls attention to impairment of concentration in severe cases of acute nephritis, but does not give any cases to demonstrate it.

Goldring, Clarke, and Smith[36] showed that at low plasma levels of phenol red, about 94 per cent of the dye clearance is accomplished by tubular activity in the normal human kidney. On the basis of this work Goldring and Smith[37] have studied the glomerular and tubular functions of the kidney with inulin and phenol red clearance ratios in patients with various stages of glomerulonephritis. In general, they have found in the earlier stages of the disease that the phenol red-inulin clearance ratios tend to maintain the normal value, which is above 3.0. They have interpreted this as suggesting that injury of glomeruli and tubules progress in a parallel manner. In the more advanced cases, however, this ratio fell as low as 0.6, indicating almost complete loss of the capacity of the tubules to excrete the dye. In a few patients observed early in the acute stage of the disease, the phenol red-inulin ratio was 210 per cent of the average normal figure. In interpreting this they have suggested the possibility of dilatation of the efferent glomerular arteriole which would give a better blood supply to the tubules, and at the same time lower the effective filtration pressure in the glomeruli. They have also suggested that permeability of glomerular capillaries may be reduced without obstructing the blood flow.

COMMENTS

It may be readily understood from the anatomic relationships of the blood supply to the tubules, which is derived from the efferent glomerular arterioles, that the tubular function of concentration may suffer when the glomerular tufts are so damaged as to reduce the urea clearance to low levels. It is difficult to understand, however, why the tubular function may be impaired long after glomerular function has returned to normal. As a tentative explanation, I offer that the tubular capillaries in some instances are damaged to a great degree at the same time the glomerular tufts are damaged, the latter recovering more quickly.

It is certain that tubular function is impaired in acute glomerulonephritis. This is shown by the impaired capacity to concentrate urine, which is illustrated by the cases which have been summarized in Table I. It is further supported by the results of Goldring and Smith[37] which indicate that glomerular and tubular damage proceed in a parallel fashion.

On the basis of the reports by Holten[34] and Alving and Van Slyke[23] of cases of acute glomerulonephritis which showed a return of urea or creatinine clearances to normal levels long before the return of the concentrating capacity, I have made the suggestion that tubular function may be impaired in acute glomerulonephritis not only because the tubular blood supply is disturbed in the diseased glomeruli, but also because the tubular capillaries themselves may possibly be the seat of specific pathology, or that specific damage to the tubular cells may account for it. The specific tubular capillary or cellular damage theory gains some support in the results of Goldring and Smith which showed in advanced cases of nephritis the reduction of the phenol red-inulin clearance ratios to such low levels as 0.6.

It may be seen from Table I that when there is sufficient glomerular damage in acute nephritis to cause nitrogen retention, there is sufficient tubular damage to interfere with the concentrating function of the kidney. I have found no exception in the literature to this. It has been emphasized that it is necessary to correct specific gravity values when the urine contains large amounts of protein.

It is my belief that impairment of concentrating capacity of the kidney will be found in most of the severe cases of acute glomerulonephritis if properly evaluated.

SUMMARY

The literature has been reviewed and twenty-two cases of acute glomerulonephritis with impaired capacity to concentrate the urine have been found and summarized in tabular form, together with four additional cases of acute glomerulonephritis which showed impairment of this function. Certain theoretical considerations have been discussed.

CONCLUSIONS

It is established that the capacity of the kidney to concentrate urine in acute glomerulonephritis may be impaired.

Most, if not all, cases with sufficient glomerular damage to cause blood nitrogen retention will show impaired ability to form a concentrated urine.

Nitrogen retention may occur in acute glomerulonephritis with less impairment of concentration than is usually observed with nitrogen retention in chronic glomerulonephritis.

The necessity for making corrections in the specific gravities of urines which contain large amounts of protein is emphasized.

The cause of impaired tubular function in acute glomerulonephritis is due, at least in part, to the interference with tubular blood supply produced by the proliferative and exudative processes in the glomeruli.

There is evidence that there may be a specific localized effect of the toxic agent of acute glomerulonephritis on the capillaries or cells of the tubules.

REFERENCES

1. Van Slyke, D. D., Stillman, Edgar, Moller, Eggert, Ehrlich, W., McIntosh, J. F., Leiter, L., Mackay, E. M., Hannon, R. R., Moore, N. S., and Johnston, Christopher: Observations on the Courses of Different Types of Bright's Disease, and on the Resultant Changes in Renal Anatomy, Medicine 9: 257, 1930.
2. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Interpretations, Baltimore, 1932, Williams & Wilkins Co., Vol. 1, p. 354.

3. Addis, T., and Shevky, M. C.: A Test of the Capacity of the Kidney to Produce a Urine of High Specific Gravity, Arch. Int. Med. **30**: 559, 1922.
4. Lashmet, F., and Newburgh, L. H.: The Specific Gravity of the Urine as a Test of Kidney Function, J. A. M. A. **94**: 1883, 1930.
5. Lundsgaard, C.: Modification of the Concentration Dilution Test, 1920 (Unpublished). Quoted by Alving, Alf S., and Van Slyke, D. D.: The Significance of Concentration and Dilution Tests in Bright's Disease, J. Clin. Investigation **13**: 969, 1934.
6. Mattice, Marjorie R.: Chemical Procedures for Clinical Laboratories, Philadelphia, 1936, Lea & Febiger, p. 220.
7. Kolmer, John A., and Boerner, Fred: Approved Laboratory Technic, ed. 2, New York, 1938, D. Appleton-Century Co., p. 132.
8. Lashmet, F. H., and Newburgh, L. H.: An Improved Concentration Test of Renal Function, J. A. M. A. **99**: 1396, 1932.
9. Emerson, Charles Phillips: Textbook of Medicine, Philadelphia, 1936, J. B. Lippincott Co., p. 976.
10. Cecil, Russel L.: A Textbook of Medicine, Philadelphia, 1938, W. B. Saunders Co., p. 946.
11. Meakins, Jonathan Campbell: The Practice of Medicine, St. Louis, 1938, The C. V. Mosby Co., p. 1162.
12. Boyd, William: A Textbook of Pathology, Philadelphia, 1938, Lea & Febiger, p. 642.
13. Best, Charles H., and Taylor, Norman B.: The Physiological Basis of Medical Practice, Baltimore, 1937, William Wood & Company, p. 620.
14. Ibid., p. 634.
15. Christian, H. A., and O'Hare, J. P.: Oxford Medicine, New York, Oxford University Press, Vol. III, Part II, p. 668.
16. Todd, J. C., and Sanford, A. H.: Clinical Diagnosis by Laboratory Methods, Philadelphia, 1936, W. B. Saunders Co., p. 198.
17. Musser, J. H.: Internal Medicine, ed. 3, Philadelphia, 1938, Lea & Febiger, p. 582.
18. Osler, Sir William: The Principles and Practice of Medicine, New York, ed. 13, revised by H. A. Christian, D. Appleton-Century Co., p. 865.
19. Boyd, William: The Pathology of Internal Diseases, ed. 2, Philadelphia, 1935, Lea & Febiger, p. 433.
20. Bell, E. T.: The Pathology and Pathogenesis of Clinical Acute Nephritis, Am. J. Path. **13**: 497, 1937.
21. Hou-Jensen, H. M.: Die Verästelung der Arteria renalis in der Niere des Menschen, Ztschr. f. d. ges. Anat. (Abt. 1) **91**: 1, 1929.
22. Clara, M.: Anatomie und Biologie des Blutkreislaufes in der Niere, Arch. f. Kreislaufforsch. **3**: 42, 1938.
23. Alving, Alf S., and Van Slyke, D. D.: The Significance of Concentration and Dilution Tests in Bright's Disease, J. Clin. Investigation **13**: 969, 1934.
24. Albright, Fuller: In Discussion of Case Records of the Massachusetts General Hospital, edited by T. B. Mallory, New England J. Med. **217**: 274, 1937.
25. Ibid. **213**: 1030, 1935.
26. Murphy, F. D., Grill, John, and Moxon, G. F.: Acute Diffuse Glomerular Nephritis, Arch. Int. Med. **54**: 483, 1934.
27. Addis, Thomas, and Oliver, Jean: The Renal Lesion in Bright's Disease, New York, 1931, Paul B. Hoeber, pp. 51, 77, 81, 229.
28. Rehberg, P. B.: The Kidney in Health and Disease, by Berglund, Hilding, Medes, Grace, Huber, G. C., Longcope, W. T., and Richards, A. N., Philadelphia, 1935, Lea & Febiger, p. 86.
29. Longcope, W. T.: Ibid., p. 362.
30. Volhard, F., und Fahr, T.: Die brightsche Nierenkrankheit, Berlin, 1914, Julius Springer, pp. 119, 125.
31. Volhard, F.: Handbuch der inneren Medizin. Nieren und ableitende Harnwege, edited by von Bergmann, G., von Staehelin, R., and others, Band VI, Teil 2, Berlin, 1931, Julius Springer, p. 1510.
32. Donath, Julius: Ueber postinfektiöse Schädigungen der Nierenfunktion, Wien. klin. Wchnschr. **43**: 591, 1930.
33. Holten, Cai: Observations Regarding Kidney Function Tests in Acute Nephritis, Am. J. M. Sc. **185**: 789, 1933.
34. Holten, C., and Rehberg, P. B.: Studies on Pathological Function of Kidneys in Renal Disease, Especially Bright's Disease, Acta med. Scandinav. **74**: 479, 1931.
35. Lyttle, John D.: The Treatment of Acute Glomerulonephritis in Children, Bull. New York Acad. Med. **14**: 212, 1938.
36. Goldring, W., Clarke, R. W., and Smith, H. W.: The Phenol Red Clearance in Normal Man, J. Clin. Investigation **15**: 221, 1936.
37. Goldring, W., and Smith, H. W.: Differentiation of Glomerular and Tubular Function in Glomerulonephritis, Proc. Soc. Exper. Biol. & Med. **37**: 180, 1937.

BLOOD CHEMISTRY DETERMINATIONS IN PERNICIOUS ANEMIA*

EVANS W. PERNOKIS, M.D., AND M. R. FREELAND, PH.D.
CHICAGO, ILL.

THE object of this paper is to report the blood chemistry determinations on 15 patients with pernicious anemia in a state of relapse. These were carried out with the hope that some alteration in the normal constituents of the body metabolism might be revealed which would aid in elucidating the obscure nature of this disease.

Minot and Murphy discovered that patients with pernicious anemia improved when given liver. Castle and his associates showed that patients with untreated pernicious anemia lacked in their gastric secretions an essential thermolabile substance termed the intrinsic factor. Sturgis and Isaacs later demonstrated the effectiveness of pig stomach in the treatment of pernicious anemia, and the similarity of this active principle to that found in normal human gastric juice. Both of these factors differ from the substance found in the liver, which is able to stimulate the bone marrow. It is assumed that the intrinsic factor found in the gastric juice combines with an extrinsic factor present in beef to form the active principle which is stored in the liver and is able to stimulate the bone marrow to normal activity. Dakin and West have demonstrated a substance in liver which is probably a dipeptide of b-hydroxyglutamic acid and l-γ-hydroxyproline which they believe to be the active principle in the liver. It was for some time believed that the extrinsic factor was the vitamin B_2 or G, which has been shown to be riboflavin. Mixtures of riboflavin and normal gastric juice, when administered to patients with untreated pernicious anemia, failed to elicit a reticulocyte response, and it is doubtful whether riboflavin is the extrinsic factor.

Even though we have a treatment for pernicious anemia, and have made progress in locating the site and the nature of the active principle, there is still much to be learned about the nature of the disease, and the active principle involved in its production. It is with this hope that we present the following data. All the determinations have been done in the laboratories of the Presbyterian Hospital of Chicago, and references to the methods used will be found in the bibliography.

Table I contains all the results on lipoid metabolism we were able to obtain. We are cognizant of the fact that Peters and Van Slyke, in the chapter on lipoids, express the opinion that increased lipoids in the various types of anemia is merely an expression of a general reaction to loss of blood and hemoglobin from the circulating fluid. This reaction is said to consist of an increase

*From the Department of Internal Medicine, Rush Medical College, University of Chicago, and the Laboratories of the Presbyterian Hospital.
Received for publication, June 1, 1940.

of fat and fatty acids in the blood, and a simultaneous deficiency of the phospholipids and cholesterol. A summary of the results reveals the following:

1. Thirteen total lipoid determinations done on 9 patients showed values twice that of normal or higher.

2. Sixteen total cholesterol determinations done on 12 patients demonstrated normal values in 6, moderately decreased in 9, and markedly decreased in 9. The distribution between the free and esterified form was essentially normal.

3. Lipoid phosphorus and lecithins were markedly diminished in 16 determinations done on 12 patients.

4. Of 11 fatty acid determinations done on 8 patients, 7 were much below minimal values, and 4 were below normal values.

TABLE I

CASE NO.	HB.	R.B.C.	W.B.C.	TOTAL LIPIDS (GM. %)	TOTAL CHOLESTEROL	FREE CHOLESTEROL	CHOLESTEROL ESTERS	LIPOID PHOSPHORUS	LECITHINS	FATTY ACIDS	MATERIAL USED
						MG. PER 100 C.C.					
1	35	1.540	5.6	1.2	158.7	54.3	104.4	5.2	130		Plasma
2	40	1.170	3.7	1.29	125.7	71.4	54.3	3.3	82.5		Plasma
3	40	1.480	4.8		116	50	66	5.4	129		Plasma
4	31	1.117	3.6	2.8	119	61	58	6.3	156		Plasma
5	58	2.350	3.6		261	92	169	12.1	303	419	Plasma
5	58	2.350	3.6		253	110	143	11.6	289		Serum
6	55	2.080	5.8	1.89	222	128	94	9.2	229	338	W.B.
6	55	2.080	5.8	1.43	234	118	116	7.4	185	337	Serum
6	55	2.080	5.8	1.43	205	104	101	7.3	184	336	Plasma
7	55	1.620	5.4	1.50	178	84	94	8.4	211	133	Serum
7	55	1.620	5.4	1.50	222	127	95	7.9	179	142	Plasma
8	56	2.320	5.6	1.69	157	67	90	8.0	200	256	Plasma
9	68	2.800	8.7	1.53	174	65	109	12.7	318	285	Plasma
10	28	1.280	3.2	1.73	161	73	88	8.2	205	244	Plasma
11	36	1.280	4.3	1.53	180	81	99	14.9	372	163	Plasma
12	40	1.800	3.8	1.29	93	43	50	6.3	157	211	Plasma

TABLE II

CASE NO.	HB.	R.B.C.	W.B.C.	AMINO ACIDS	Ca	P	N.P.N.	UREA N	URIC ACID	CREATININE	SUGAR	CO_2	NaCl
1	35	1.54	5.6	11.7									
2	40	1.17	3.7	10.1									
3	40	1.48	4.8	5.9									
4	31	1.12	3.6	5.7									
6	55	2.08	5.8	4.8	10	4	27.8	10	3	1.2	93	69.9	495
8	56	2.32	5.6		10	3.9	25	11	2.7	1.1			550
9	68	2.80	8.7		8.7	3.1	29				113		
10	28	1.28	3.2		8.4	3.5	44.4	23.5	3.6			55	530
13	30	0.98	4.8	21.5									

Table II shows the results of a smaller series of blood chemistry determinations done on 9 patients.

1. Amino acid determinations done on 6 patients showed normal values in 3, slightly elevated values in 2, and a value of 21 mg., which is nearly three times normal, in one.

2. Calcium and phosphorus determinations on 4 patients were normal as were two creatinine and three uric acid determinations.

3. Four nonprotein nitrogen determinations were done, 3 of which were normal and one slightly elevated. Two urea nitrogen determinations were normal, and the third, the same patient who showed the increase in the nonprotein nitrogen, showed also an increase in the urea nitrogen.

4. Two glucose determinations, as well as two carbon dioxide, and three sodium chloride determinations were normal.

TABLE III

CASE NO.	HB.	R.B.C.	W.B.C.	ROSE BENGAL* (%)		INORGANIC SULFUR	ETHEREAL SULFUR	TOTAL PROTEIN	ALBUMIN	GLOBULIN	A./G.
				10 MIN.	18 MIN.	(MG. %)					
1	35	1.54	5.6	63.6	45.4						
2	40	1.17	3.7	62.5	50.0						
4	31	1.12	3.6	35	25						
13	30	0.98	4.8	56	40	3.6	0.6				
14	45	1.34	3.0	66.6	55.5	2.9	0.8				
6	55	2.08	5.6					8.41	4.63	3.73	1.22
9	68	2.80	8.7					5.5	3.76	1.74	2.16
10	28	1.28	3.2					6.27	4.15	1.18	1.95

*Normal values: 10 min., 55 per cent or less; 18 min., 35 per cent or less.

Table III shows the results of 5 Rose Bengal liver function tests, 3 total protein and albumin and globulin determinations, and 2 sulfur determinations.

1. The liver function tests showed dye retention in 4 of 5 determinations.

2. The blood protein determinations done on 3 patients were within normal values as was the distribution between the albumin and globulin fractions.

3. Inorganic and ethereal sulfates of the blood were found normal in 2 patients.

TABLE IV

CASE NO.	HB.	R.B.C.	W.B.C.	B.M.R.	ICTERIC INDEX	TEMPERATURE
1	35	1.54	5.6	plus 28	18	99
2	40	1.12	3.7	plus 13	15.2	98.8
3	40	1.48	4.8	plus 30	16	99
4	31	1.12	3.6	plus 21	7	98.8
5	58	2.35	3.6	0	20	98.2
6	55	2.08	5.8	plus 17	15	99.4
7	55	1.62	5.4	plus 6	7.1	98.4
8	56	2.32	5.6	plus 12	8	98.2
9	68	2.80	8.7	plus 15	5.7	98.2
10	28	1.28	3.2	plus 22	21.4	99.4
11	40	1.20	4.3	plus 9	22	99.2
12	40	1.80	3.8	plus 18	19	98.6
13	30	0.90	4.8	plus 32	33	98.6
14	45	1.34	3	plus 28	18	99.2
15	54	1.85	3.8	plus 20	13.3	98.2

Table IV shows the basal metabolic rates of 15 patients and their icteric indices. The temperatures are included merely to indicate that at the time the basal metabolic rates were taken patients had no significant temperature.

1. Three of the 15 basal metabolic rate determinations only were normal, the other 12 being definitely elevated.

2. The icteric index determinations were normal in 4 of 15 patients on whom they were done.

SUMMARY

1. Total lipoids on all determinations were increased much above normal values. The fatty acids were very much diminished in all the determinations, as were the "lecithins" and the lipoid phosphorus. Cholesterol seemed normally distributed between the free and esterified forms, and showed values that were normal or slightly diminished. Only one cholesterol determination was below 100 mg.

2. One of 6 amino acid determinations showed a marked increase, while the other 5 were within normal values.

3. Calcium, phosphorus, uric acid, creatinine, sugar, chlorides, and carbon dioxide determinations were found normal. One nonprotein nitrogen and one urea nitrogen determination showed an increase above normal.

4. Total protein, albumin, and globulin values on 3 patients were normal.

5. Inorganic sulfates and ethereal sulfates on 2 patients were normal.

6. Four Rose Bengal liver function tests showed dye retention, and the fifth was normal.

7. The icteric index was increased in 73.3 per cent of the cases.

8. The basal metabolic rate was increased in 80 per cent of the cases.

9. One glutathione determination done on a patient who had hemoglobin 16 per cent and a red blood cell count of 0.710 showed 14.5 mg. per cent of reduced glutathione and none of the oxidized. The patient died two days later; a section of liver taken from the autopsy and extracted with saline showed 168.7 mg. of reduced glutathione per 100 Gm. of liver. No oxidized glutathione was demonstrated in the saline liver extract.

NOTE.—Blood lipoid determinations were all done by the Bloor method and were begun before the work of Christensen[10] and Van Slyke and Folch[11] was published. This study of the lipoids in pernicious anemia is being continued in our laboratories by both the Bloor method and the modifications suggested by the authors mentioned.

REFERENCES

1. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Baltimore, 1931, Williams & Wilkins Co., Vol. I.
2. Mathews, A. P.: Principles of Biochemistry, Baltimore, 1937, William Wood & Co.
3. Casele, W. R., and Minot, G.: Pathological, Physiological and Clinical Description of the Anemias, New York, 1936, Oxford University Press.
4. Naegeli, O.: Blutkrankheiten und Blutdiagnostic, ed. 5, Berlin, 1931, Julius Springer.
5. Clough, P. W.: Diseases of the Blood, New York, 1929, Harper & Brothers.
6. Bloor, W. R.: Total Lipoids, J. Biol. Chem. **17**: 377, 1914.
7. Bloor, W. R.: Fatty Acids, J. Biol. Chem. **77**: 53, 1928.
8. Bloor, W. R., Pelkam, K. F., and Allen, D. M.: Total Cholesterol, J. Biol. Chem. **52**: 191, 1922.
9. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Extraction of Lipoids, p. 884; Determination of Phosphorus, p. 882, Baltimore, 1932, Williams & Wilkins Co., Vol. II.
10. Christensen, H. N.: Contaminants of Blood Phospholipids, J. Biol. Chem. **129**: 531, 1939.
11. Van Slyke, D. D., and Folch, J.: Nitrogenous Contaminants of Petroleum Ether Extracts, J. Biol. Chem. **129**: 539, 1939.

LABORATORY METHODS

GENERAL

A SIMPLE NONTRAUMATIZING METHOD OF INDUCING PNEUMOCOCCIC INFECTION IN ALBINO RATS*

ELMER H. LOUGHLIN, M.D., SAMUEL H. SPITZ, M.D., AND
RICHARD H. BENNETT, M.D., BROOKLYN, N. Y.

WITHIN the past few years there have been developed new therapeutic agents for use in the treatment of pneumonia. Since there is great variability in the severity of human pneumococcic infections from year to year, it is necessary before these substances are used clinically to determine first their efficacy and toxicity under controlled conditions.

Tests in vitro partially demonstrate the efficacy, but obviously give no clue as to the toxicity of these preparations. To determine their toxicity and complete effectiveness in infection, experimental animals must be used.

It is well known that albino rats can be infected with pneumococci. Intraperitoneal inoculation and intrabronchial instillation of pneumococci are two procedures which have been used to infect them. The former causes a high incidence of infection and the dose of organisms can, with reasonable accuracy, be controlled. This method, however, does not reveal the effectiveness of therapy when there is pulmonary involvement.

With the latter method, originated by Nungester and Jourdonais,[1, 2] and used by Gross and Cooper,[3, 4] in the study of antipneumococcic drugs, in which pneumococci are suspended in gastric mucin, and instilled into a bronchus, infection is irregular and the recovery rate of the untreated or control animals is too high to warrant accurate evaluation of the therapeutic agent. Even though carefully performed, this procedure causes trauma which interferes with the interpretation of therapeutic efficacy and incidentally the pathogenesis.

A method, therefore, is required which, with a minimum of trauma, will produce a high incidence of pneumococcic infection with pulmonary involvement, and a constantly high fatality rate in infected animals. With these facts in mind, the following procedure is suggested.

METHOD

An unanesthetized rat, weighing about 300 Gm., is fastened with rawhide thongs to a small animal board (Fig. 1) designed for this procedure. A wire loop, attached to the undersurface of the board with a rubber band, is slipped

*From the Department of Internal Medicine, Long Island College of Medicine.

This work was made possible by a Fellowship Grant from the Lilly Research Laboratories, Indianapolis, Ind., and by the Dr. Frank E. West Fund, established by Miss Elizabeth Frothingham, and the John C. Warren Memorial Fund, established by Dr. Luther F. Warren, in memory of his son.

Received for publication, March 23, 1940.

over the animal's upper incisor teeth, and a strip of gauze is placed over the lower incisor teeth to pull the jaw open.

A pneumococcus culture, consisting of the washings of the peritoneal cavity of a white mouse infected with pneumococci,* is suspended in 15 c.c. of a physiologic solution of sodium chloride. This is sprayed into the pharynx and nares of the rat by means of an atomizer, the bulb being squeezed four times. This culture contains, as a result of the peritoneal inflammation, natural mucin in addition to the other components of an inflammatory exudate.

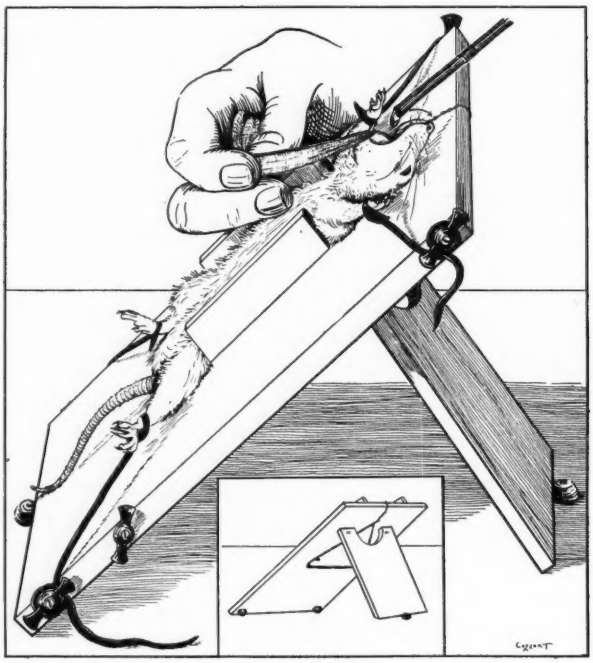


Fig. 1.

After preliminary treatment with soap and water, followed by acetone and alcohol, blood for culture is obtained from the tail of the animal by snipping off the tip with a pair of sterilized scissors. The drops of blood appearing at the cut end are placed directly on a blood agar plate and then streaked over the surface with a wire loop.

RESULTS

Of 131 animals thus sprayed 107, or 81.6 per cent, became infected as manifested by positive blood cultures.

Only those animals in which a growth of pneumococci in the blood culture was obtained were considered as having been infected. These animals invariably died. Although positive cultures have been obtained as early as four hours, the majority of the animals developed positive cultures between six and twenty-four hours after spraying.

*RI strain pneumococci were used in all experiments.

In the animals that died of pneumococcic infection, patches of pneumonia, sometimes involving an entire lobe or multiple lobes, were noted. In the majority of these animals this patchy pneumonic process was associated with empyema and purulent pericarditis. A purulent peritonitis sometimes developed and, in a few, meningitis occurred.

When the rats died of any of the above conditions a growth of pneumococci was obtained from the heart's blood.

DISCUSSION

The method of infecting rats with pneumococci in which pulmonic infection and bacteriemia were common is nontraumatic, easily and rapidly performed, and permits, when necessary, large numbers of animals to be used for therapeutic studies.

Many investigators have sprayed the upper respiratory tract of animals attempting to produce pneumococcic pulmonary infections, but the results were generally unsatisfactory. In these cases the pneumococci used were grown in artificial media. Pneumococcic pneumonia in man is most frequently caused by pneumococci which have recently been in contact with a human host or carrier. It is known that organisms recently in contact with an animal host are more virulent than those grown in artificial media from eighteen to twenty-four hours. In order to simulate as closely as possible the conditions in human infection, when attempting to infect rats, we have used pneumococci in mouse peritoneal exudate, containing natural mucin, because this procedure appears to satisfy this requirement best. Not only are these organisms more virulent, but, as pointed out by Nungester and Jourdonais,[2] gastric mucin, when inoculated intrabronchially with pneumococci, hinders or prevents rapid phagocytosis of these organisms by the leucocytes of the infected animals. Such conditions probably exist in human infection.

SUMMARY

1. A simple nontraumatizing method of infecting rats with pneumococci has been described, in which pneumococcic exudate from the peritoneal cavity of the mouse is used because of the increased virulence of pneumococci recently in contact with an animal host and because of the natural mucin content of the exudate.

2. A high incidence of infection, 81.6 per cent, was produced as manifested by positive blood cultures, and at autopsy, by pneumonia, empyema, purulent pericarditis, peritonitis, or meningitis.

We wish to express our appreciation to Doctors Tasker Howard, J. Hamilton Crawford, Wade W. Oliver, and Arnold Eggerth for their aid and criticism in the preparation of this paper.

REFERENCES

1. Jourdonais, L. F., and Nungester, W. J.: Intratracheal Inoculations in the Rat, Science **81**: 74, 1935.
2. Nungester, W. J., and Jourdonais, L. F.: Mucin as Aid in Experimental Production of Lobar Pneumonia, J. Infect. Dis. **59**: 258, 1936.
3. Gross, P., and Cooper, F. B.: Efficacy of p-Aminobenzenesulfonamide in Experimental Type III Pneumococcus Pneumonia of Rats, Proc. Soc. Exper. Biol. & Med. **36**: 225, 1937.
4. Gross, P., and Cooper, F. B.: P-Aminobenzenesulfonamide and Antipneumococcal Serum Therapy in Type I Pneumococcal Infections of Rats, Proc. Soc. Exper. Biol. & Med. **36**: 535, 1937.

ORAL AND NASAL SIMULTANEOUS ASPIRATORS AND THEIR USE IN GASTROENTEROLOGY

MOSES EINHORN, M.D., NEW YORK, N. Y.

THE duodenum does not play a very important part in the physiology of digestion, except that it receives, aside from its own secretion, the combined secretions of the pancreas, liver, and biliary tract. It is here that the material coming from the stomach meets these various secretions. Thus the duodenum serves not only as a melting pot, but also as a conduit for conveying the combined material into the jejunum and ileum where the final process of digestion occurs.

Due to its position investigators made numerous attempts in the past to intubate the duodenum, among the first of whom were Hemmeter and Kuhn. The instruments they employed were crude and impractical and were therefore abandoned.

Max Einhorn experimented for a number of years with various tips and buckets, and finally, in 1909, developed the first practical apparatus which he called the duodenal pump.[1] Since that time eminent gastroenterologists have developed various types of tips, each claiming particular advantages, but all alike in principle, differing only in their size, weight, and number of perforations (Fig. 1).

In 1938 I developed a bucketless tube,[2] a radical departure from the other tubes in use. The apparatus is 50 inches in length (Fig. 2), 14 French in diameter, and its terminal end encases an elongated lead dropper, narrow and tapered above, but increasing in size below. Two openings, slotted in appearance, are situated just above the dropper, ½ inch apart, on opposite sides of the tube.

The advent of the duodenal tube enabled physiologists, clinicians, and research workers to make a comprehensive survey and study of the functions of the stomach, liver, and pancreas. Yet, in their research work, they still felt the growing need for an instrument which would intubate both the stomach and duodenum, and permit a simultaneous withdrawal of their respective contents.

Max Einhorn[3] in 1918 demonstrated an apparatus (Fig. 3*A*) by means of which the gastric and duodenal contents could be obtained at the same time. His aspirator consisted of a long soft rubber tube, about 12 French in diameter, divided into two separate canals, one leading to a terminal perforated capsule and the other to a perforated hollow metal connector situated about 25 cm. from the end of the tube. The proximal end of the tube branched off into two side tubes each provided with stopcocks, one marked *D* for the duodenum and the other *S* for stomach.

Received for publication, April 10, 1940.

This double aspirator was provided with markings starting from the terminal end of the tube, and was introduced in the same manner as the usual duodenal tube, so that while the bucket entered the duodenum, the perforated connector lay within the limits of the stomach, thereby allowing the duodenal contents to be aspirated separately through the terminal tip and the gastric contents through the perforated connector.

For the simultaneous sampling of the gastroduodenal contents, Rehfuss first employed a method in which he used two separate tubes of different lengths. The patient first swallowed the longer tube in the usual way, and then, after it was ascertained that the tip had entered the duodenum, he swallowed the shorter tube.

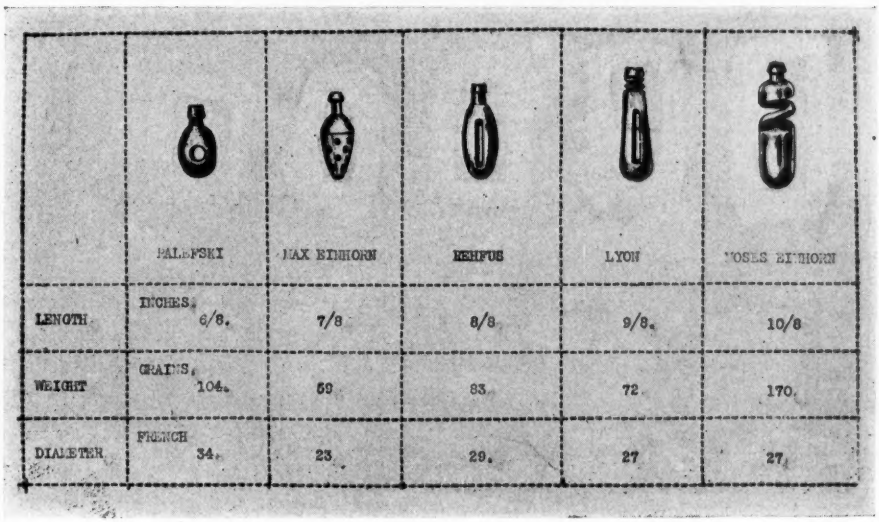


Fig. 1.—Various tips used in gastrointestinal work.

To simplify it further, Rehfuss employed a second method[4] whereby he attached the capsule of the shorter tube to the wall of the longer tube (Fig. 3*B*) at a distance of about 25 cm. from its end, so that when the distal tip was in the duodenum, the proximal capsule lay within the limits of the stomach.

In 1927 Kunstler, impressed by the findings of Garbat[5] and Carlson[6] that duodenal feeding promotes a rise of gastric acidity, suggested that constant and complete neutralization of gastric acidity in duodenal feedings could be accomplished by administering an alkaline solution by means of the continuous drip method. He designed for that purpose a special double tube.[7] This consisted of an ordinary tube (Fig. 3*C*) to whose wall was vulcanized at a point 20 cm. from its bucket a shorter tube, smaller in caliber and open at its end for a distance of 70 cm., thus leaving the remaining 20 cm. free. His idea of treatment was to administer two-hour duodenal feedings through the longer tube and introduced a weakly alkaline solution for the full twenty-four hours by the drip method through the shorter tube.

The well-known Bayliss and Starling[8] discovered the substance secretin which is formed when the acid contents of the stomach pass into the duodenum, which in turn, stimulates the production of pancreatic secretion and its enzymes.

This work has been confirmed in its major aspects by most physiologists and, hence, secretin has been the substance used when a substantial increase in the volume of the pancreatic secretion was desired.

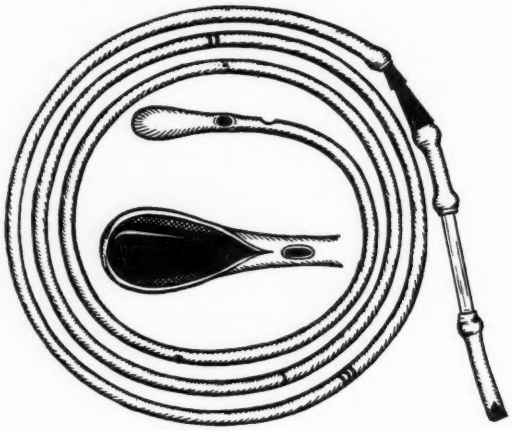


Fig. 2.—Einhorn's bucketless lead weighted gastroduodenal tube.

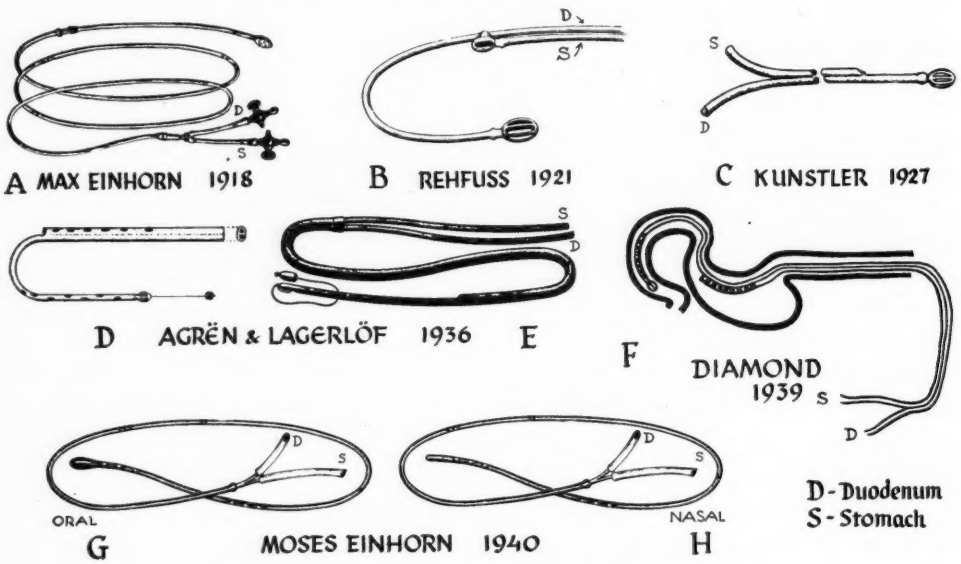


Fig. 3.—Various simultaneous gastroduodenal aspirators.

The Swedish investigators, Ågren and Lagerlöf,[9] made an extensive study of pancreatic secretion in man after intravenous administration of secretin and designed a special double tube for that purpose (Fig. 3*D*, *E*). Their tube consisted of two tubes free for the first 20 cm. and fused together for a distance of 70 cm. The duodenal side, which extended for a distance of 20 cm. and had a capsule at its end, featured seven oval-shaped openings on its last 8 to 10 cm. of tubing. The gastric tube had five to six openings on its side. The whole of this 70 cm. of fused tubing was encased in a thin rubber sheath to facilitate swallowing. In order to accelerate the entrance of the duodenal tube into the duodenum, they tied to its end a fine silk cord, 10 cm. in length,

to which was attached a lead ball 8 mm. in diameter. The tube was swallowed in the usual way, and when the tip entered the duodenum, the openings of the gastric tube remained in the stomach area.

In this country, Diamond[10] recently made extensive studies on the use of secretin as a clinical test of pancreatic function and used a specially designed gastroduodenal tube. His apparatus permitted the separate collection of gastric and duodenal contents by preventing the admixture of the two secretions, thus assuring the collection of pure duodenal juice (Fig. 3*F*).

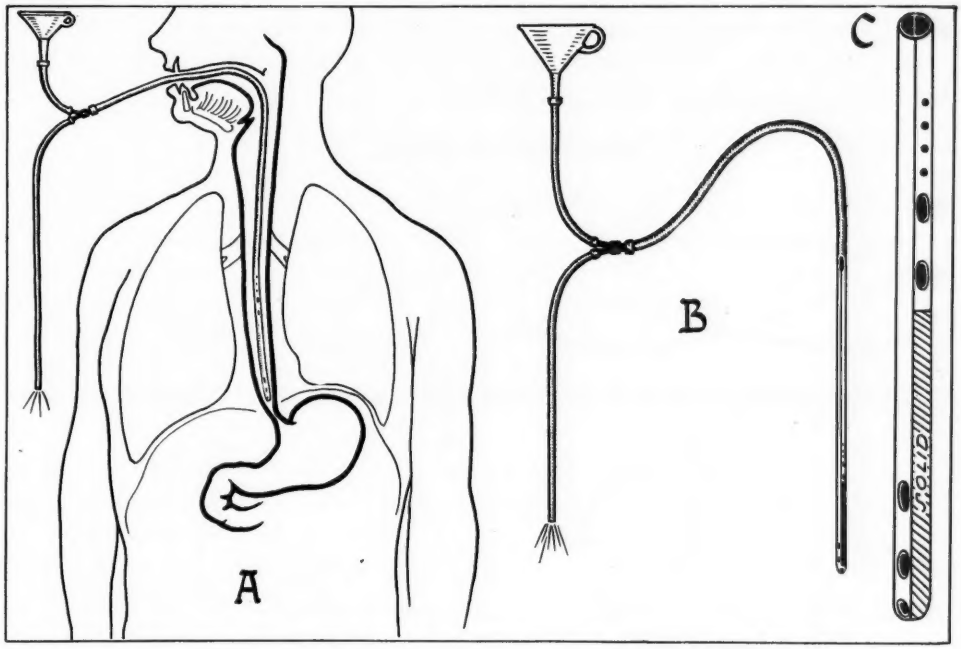


Fig. 4.—Einhorn's double channel esophageal lavage tube.

In my article on "The Treatment of Cardiospasm,"[11] I described a double lumen tube (Fig. 4) for esophageal lavage. Its basic principle is the same as that which has for some time been used in colonic irrigation and which has subsequently been adopted by Miller and Abbott for intestinal intubation. The tube is 28 French in diameter, 30 inches in length, and is bisected into two distinct canals. One of the canals terminates at a point four inches short of the tip, while the remainder of its lumen is filled with solid rubber. Immediately above this solid portion the walls feature a few wide openings. The second canal extends to the very end of the tube and has at its terminal end a few oval perforations.

The idea occurred to me that by combining the main features incorporated in the bucketless and double lumen esophageal tube, I would have a very simple and efficient either oral or nasal simultaneous gastroduodenal aspirator (Fig. 3*G, H*).

The oral tube* (Fig. 5*F*) is 50 inches in length, 16 and 18 French in diameter, semisoft in consistency, flexible, and of good resiliency. Its lumen

*Made by Clay-Adams Co.

is equally divided into two channels (Fig. 5*A*), semilunar in shape. Its terminal end, 1 inch in length, encases an elongated drop-shaped lead sinker (Fig. 5*Ga*), narrow and tapered above, but increasing in size at the bottom, this portion including the sinker with its layer of rubber being 150 grains in weight, 1 inch in length, and 27 French in diameter at the bottom, the top being of the same size as the tube. The duodenal side of the double channel extends down to the top of the sinker and has three oval openings slotted in appearance and ½ inch apart. The small space between the sinker and the lower opening is filled with rubber. The gastric side of this double channel ends at a point about 7 inches above the sinker; the remainder of its lumen is filled with lead shot (Fig. 5*Gb*). Immediately above the lead-filled portion, there are three openings, oval in shape and ½ inch apart.

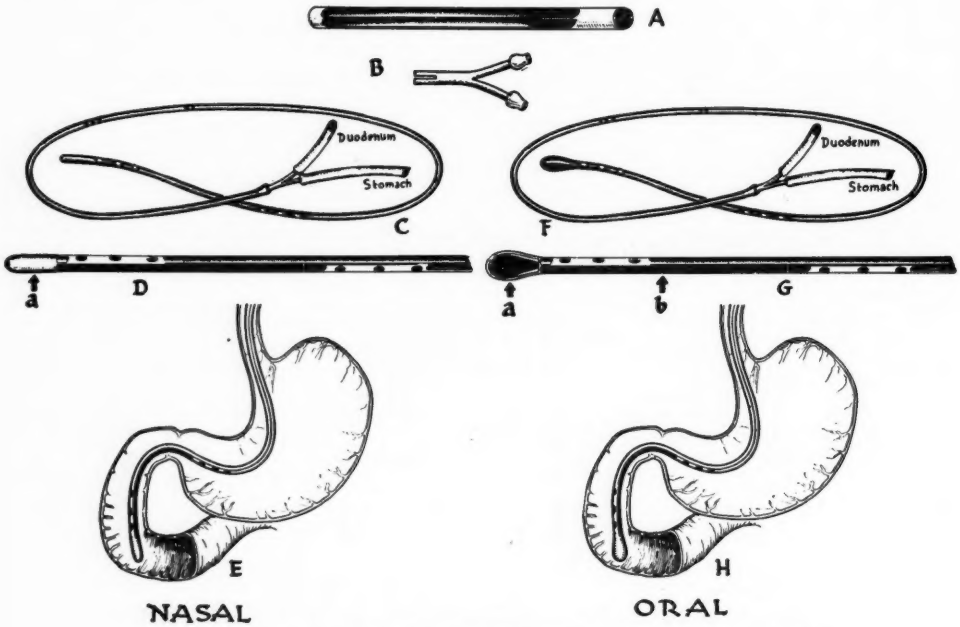


Fig. 5.—Einhorn's nasal and oral simultaneous gastroduodenal aspirators.

The nasal tube (Fig. 5*C*) is designed in the same manner as the oral, except that it is 16 French in diameter and terminates in a catheter-like tip (Fig. 5*Da*), which is made of solid impregnated lead rubber for 2½ inches.

For the oral tube the markings are as follows: the one-ring mark is placed 17 inches from the end of the tube, and represents the distance from the lips to the cardia; the two-ring mark, at 28 inches, represents the distance from the lips to the pylorus; and the three-ring mark, at 33 inches, permits 5 inches of the terminal end of the tube to enter the duodenum (Fig. 5*H*) and leaves a space of 3 inches from the lower opening of the gastric channel.

In the case of the nasal tube the one-ring mark is placed at 19 inches, the two-ring mark at 30 inches, and the three-ring mark at 36½ inches. It is understood, however, that the physician's judgment should always determine the amount of tubing for each individual and he should not rely entirely on the markings.

The special double channel connector (Fig. 5*B*) consists of two narrow metal tubes soldered together in the middle, branching off distally into two distinct separate canals, each of which is cemented into its proximal opening of the tube to render it airtight. Proximally, the connector bifurcates into two channels, one representing the gastric outlet and the other the duodenal.

ADVANTAGES

Oral Tube.—1. The tube is simple in construction and no fusion of tubes is necessary, thereby facilitating swallowing.

2. The fear the patient usually experiences that the metal bucket may become detached is eliminated, as the lead dropper is encased in the tube.

3. Swallowing of the tube is accelerated because of the weight of the dropper, which is sufficient to permit its passage through the esophagus with no discomfort to the patient.

4. The tube maintains its intended course along the stomach pathway because of the lead-shot compartment and the weight and proper arrangement of the lead sinker, which keeps it in its proper place in its progress to the pyloric opening.

5. Due to the special distribution of the weight of the sinker, which anchors the tube securely in the duodenum, there is no likelihood of regurgitation of the bucket from the duodenum into the stomach, as is usually the case with other tips.

6. No discomfort is encountered at the glottis upon removal of the tube, because of its tapered shape and gradual decrease in size at the neck.

7. There is no need for silver- or gold-plating the metal bucket to avoid impairment of the mucous membrane, since the sinker is encased in rubber.

8. The openings of the tube have rubber margins, thereby eliminating the possibility of cutting the mucous membrane upon suction, as is usually the case with metal buckets.

Nasal Tube.—1. The 2½ inch solid rubber catheter-shaped tip at the terminal end facilitates the introduction and passage of the tube through the nares.

2. The weight of the lead-impregnated rubber tip and the lead-shot compartment facilitates the passage of the tube through the esophagus, maintains it in its proper position along the stomach pathway, and aids its entrance into the duodenum.

3. There is less possibility of the lower part of the tube regurgitating into the stomach as the weight of the solid lead-impregnated rubber portion and the lead-shot compartment keeps it securely in the duodenum.

TECHNIQUE

Oral Intubation.—The patient reports on an empty stomach and is placed in an upright position on a chair or in bed, and is instructed to protrude his tongue to form a groove. The operator seizes the terminal lead sinker, previously moistened, between the thumb and forefinger, and places it on the grooved portion of the tongue. The patient is instructed to open his mouth wide and say "Ah." The operator then inserts the sinker far enough into the

mouth under the uvula and instructs him to close his mouth and concentrate on the act of swallowing. The tube, due to its lead weight, will be carried down slowly through the esophagus.

As soon as the tube has reached the first-ring mark, the patient is placed in a reclining position on the right side, with the right leg and knee stretched straight and the left leg and knee flexed, overlapping the right.

The patient is instructed to swallow the tube very slowly, an inch at a time, over a period of twenty to twenty-five minutes, until the third-ring mark is reached, so that the terminal end will enter the duodenum.

After the patient has swallowed a few inches of the tubing while lying on the right side, it is a good practice to inject a small amount of lukewarm water through the gastric canal in order to relax the pyloric opening and stimulate the peristaltic action of the stomach, thereby facilitating and accelerating the entrance of the terminal portion of the tube into the duodenum.

Upon completion of the examination, the operator withdraws the first seven inches of the tube while the patient is in a recumbent position, and the balance of the tube while the patient is in the upright position.

In case no bile is obtained, the operator should withdraw the tube up to a point midway between the first- and second-ring marks and then instruct the patient to swallow it again.

Nasal Intubation.—The terminal end of the tube, which has previously been dipped in glycerin, is introduced through the nose for a distance of six inches, and the patient is then asked to simulate the act of swallowing, while the operator pushes the tube which will be carried down slowly in the esophagus because of its lead-weight compartment.

As soon as the first-ring mark is reached, the patient is turned on the right side and the same procedure is followed as in oral intubation.

Tests for Determining the Entrance of the Terminal Tip Into the Duodenum.—1. It is possible to determine the entrance of the tip into the duodenum with the aid of fluoroscopic and x-ray examinations. Although this method is undoubtedly reliable, I believe that it should not be used in routine practice, since in the course of removal of the patient to the x-ray room and of manipulation, the terminal portion of the tube may easily slip back into the stomach even with the lead-weighted tip.

2. By observing the characteristic differences in color, viscosity, and reaction of both specimens obtained from the two outlets. The duodenal specimen will be viscid, golden yellow in color, and negative to Congo red, whereas the gastric specimen will be clearer, perhaps bile-tinged, but positive to Congo red.

3. If, after injecting a mixture of barium or any other colored solution into the duodenal outlet, no return is obtained on the gastric side, the terminal tip has entered the duodenum.

USES

Medical.—1. For obtaining a simultaneous specimen from the stomach and duodenum.

2. For determining the effect on the duodenal contents of substances introduced into the stomach and vice versa.

3. In peptic ulcer cases where duodenal feeding and intermittent or continuous neutralization by drip method is desired.

4. In cases of gastric atony, nervous dyspepsias, or any form of vomiting where duodenal gavage and gastric drainage are indicated.

5. In biliary drainage.

6. In cases where esophageal or gastric continuous lavage is required.

Surgical.—1. In preoperative and postoperative gastrointestinal and abdominal surgery, where simultaneous and continuous gastrointestinal drainage[12] is wanted.

2. In cases where tubal feeding and drainage are required.

3. In cases of gastroenterostomy.

I wish to express my gratitude to Mr. L. T. Hillborn, president of the Clay-Adams Co., for his kind cooperation in the development of the tubes.

REFERENCES

1. Einhorn, Max: A New Method of Catheterizing the Pylorus and Duodenum, New York Med. Rec. **76**: No. 15, 1909.
2. Einhorn, Moses: New Bucketless Lead Weighted Gastro-Duodenal Tube With a Review of the American Contribution to the Development of These Tubes, Am. J. Digest. Dis. **5**: 77, 1939.
3. Einhorn, Max: The Duodenal Tube, Philadelphia, 1926, F. A. Davis Co.
4. Rehfuss, M.: Diagnosis and Treatment of Diseases of the Stomach, Philadelphia and London, 1927, W. B. Saunders Co., pp. 103, 122.
5. Garbat, A. L.: Gastric Secretion in Response to Duodenal Feeding, Arch. Int. Med. **32**: 771, 1923.
6. Carlson, A. J.: Secretion of Gastric Juice in Health and Disease, Physiol. Rev. **3**: 1923.
7. Kunstler, M. B.: A New Duodenal Tube for the Treatment of Peptic Ulcer, M. J. & Rec. **125**: 466, 1927.
8. Bayliss, W. M., and Starling, E. H.: J. Physiol. **24**: 99, 1899.
9. Ågren, G., and Lagerlöf, H.: Pancreatic Secretion in Man After Intravenous Administration of Secretin, Acta. med. Scandinav. **90**: 1, 1936.
10. Diamond, J., Siegel, S., Gall, M., and Karlen, S.: The Use of Secretin as a Clinical Test of Pancreatic Function, Am. J. Digest. Dis. **6**: 366, 1939.
11. Einhorn, Moses: The Treatment of Cardiospasm Illustrating a New Cardiospasm Dilator and a Bisected Esophageal Tube, Rev. Gastroenterol. **6**: 499, 1939.
12. Einhorn, Moses: Nasal Simultaneous Gastroduodenal Aspirator: Its Use in Postoperative Gastrointestinal and Abdominal Surgery, Surg., Gynec. & Obst. **72**: 48, 1941.

A STUDENT SPHYGMOSCOPE*

ALRICK B. HERTZMAN, PH.D., ST. LOUIS, MO.

THE desirability of providing the student with laboratory experience with the type of sphygmomanometer devised by Erlanger led us some years ago to modify the sphygmoscope developed by Erlanger and Meek[1] so that advantage could be taken of apparatus available in the student equipment.

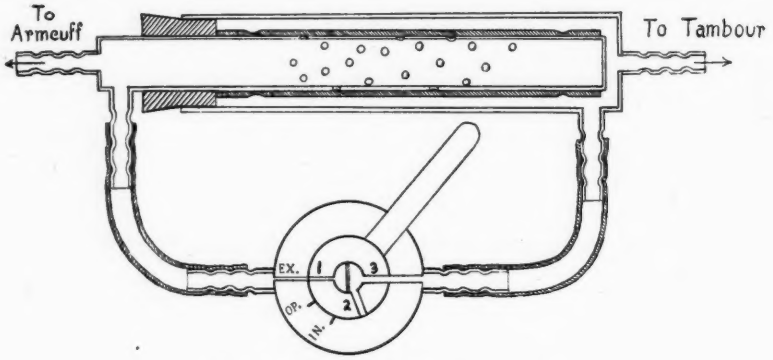


Fig. 1.—The student sphygmoscope.

The sphygmoscope shown in Fig. 1 has been in use for twelve years with a minimum of servicing by the mechanic. It is essentially the same as that of Erlanger and Meek, excepting for the arrangement of the control valve. All parts are metal. Brass tubing is used instead of glass. The rubber membrane surrounding the inner tube may be quickly made from sheet rubber of suitable thickness by cementing overlapping edges. The control valve replaces the expensive compound stopcock in the Erlanger sphygmomanometer. A capillary glass or metal stopcock placed on a side arm in the connection to the tambour provides a leak which may be easily adjusted so that it protects the tambour against slow large changes in the volume of the outer chamber of the sphygmoscope, but does not interfere with the recording of the oscillations; or a simple needle point valve may be soldered on the cup of the tambour to serve the same purpose. The sphygmoscope and control valve are mounted on a board. Overall dimensions are 8 by 4½ by 2½ inches, permitting compact storage.

Operation of Control Valve.—Three positions are available:

(1) Exhaust position (shown). Arm cuff and outer chamber of sphygmoscope are in connection with the outside through exits 1 and 3. A baffle plate in the central outlet of the cock prevents the gust of air from the arm cuff from disturbing the tambour. This position is used for deflating the arm cuff when using the intermittent escapement method. The diameter of exit 1 is

*From the Department of Physiology, St. Louis University School of Medicine.
Received for publication, April 13, 1940.

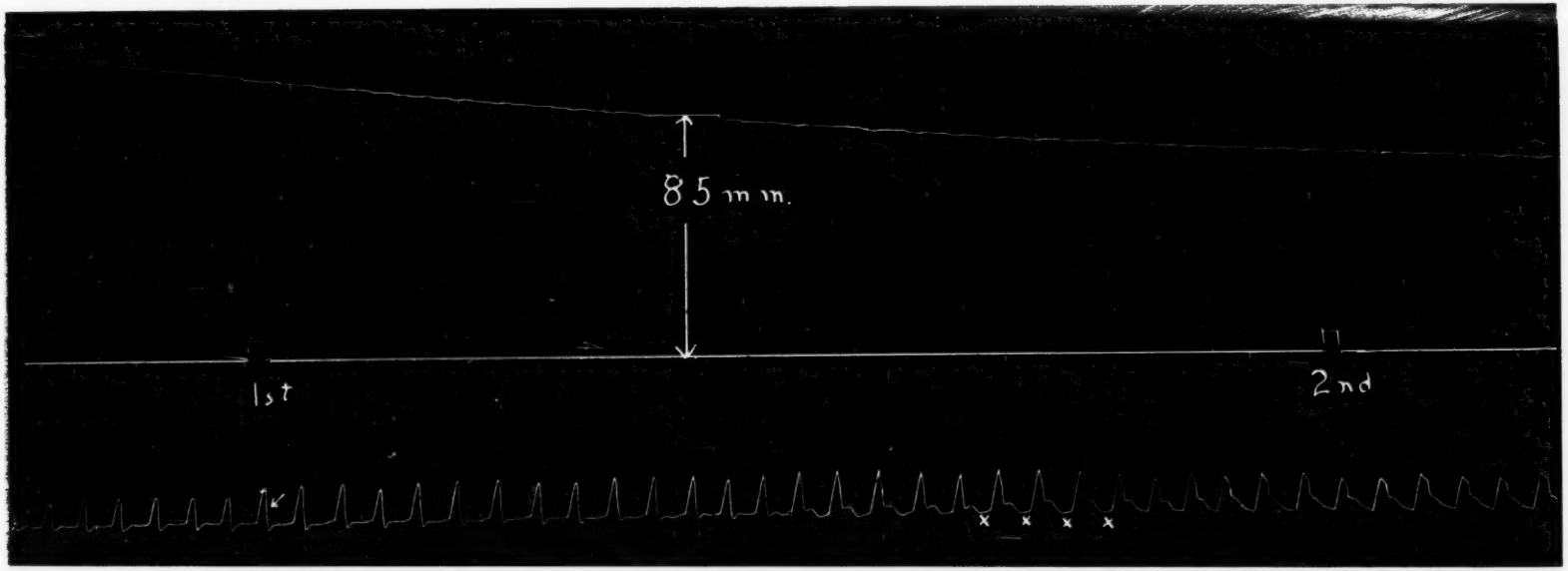


Fig. 2.—Illustrative record made with student equipment. Lower record: sphygmoscope oscillations. Note flattening in diastolic portion of curve at X, and its disappearance when diastolic pressure (auscultatory) is reached. Upper record: arm cuff pressure. Middle record: auscultatory systolic and diastolic pressures at first and second signals.

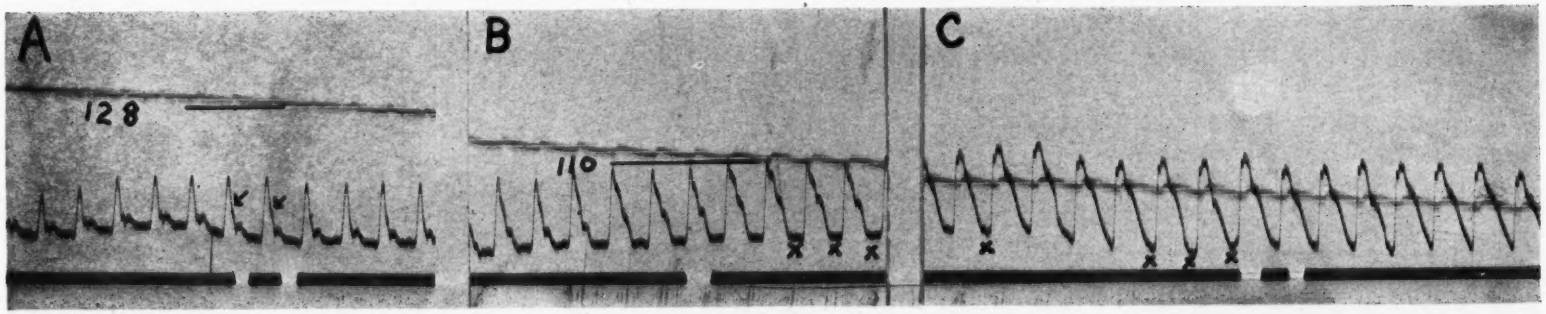


Fig. 3.—Sections from optical record. *A*, Auscultatory systolic pressure (double signal) at 128 mm. Hg. *B*, Cuff pressure 110 mm. Hg. *C*, Auscultatory diastolic pressure (double signal) at 93 mm. Hg.

made small enough so that the deflation is readily controlled. (2) Intake position. Exit 1 is in line with the scratch mark IN. Exit 2 is in connection with the outer chamber of the sphygmoscope and so prevents the large volume changes here during inflation of the arm cuff from affecting the tambour. (3) Operating position. Exit 1 is in line with the scratch mark OP. All exits are closed in this position. The oscillations of the sphygmoscope are now transmitted to the tambour. This position is used when the method of continuous escapement is employed, the tambour leak being adjusted in proportion to the rate of fall in arm cuff pressure which is controlled by the air escape valve on the cuff. The method of intermittent escapement may also be used with this position. This position is also used when one wishes to record continuously variations in arterial pressure. The sphygmoscope is very useful for student experiments of the latter type.

Criteria of Systolic and Diastolic Pressures With Sphygmoscope.—These can be studied by the student by arranging to record arm cuff pressures with a recording manometer, and signaling auscultatory systolic and diastolic criteria while the sphygmoscope oscillations are being recorded. A typical performance using a Marey tambour (Harvard Apparatus Co.) is illustrated in Fig. 3. The systolic sign, as pointed out by Erlanger, seems to be "the first clear increase in the spread of the limbs at the base of the pulse wave." The diastolic criteria of Erlanger are less apparent. Many records have shown that a flattening in the catacrotic portion of the pulse curve disappears at pressures very near those at which the Korotkow sounds become dull and muffled. This is more clearly shown in the optical records of Fig. 3. The wave is completely supported throughout the pulse cycle at lower pressures. This relationship is not entirely regular. The flattening may disappear either slightly above or slightly below the pressure at which muffling occurs.

REFERENCE

1. Erlanger, J., and Meek, W. J.: Adjustable Sphygmoscope for Recording Sphygmomanometer, J. LAB. & CLIN. MED. 12: 172, 1926.

DEXTROSE YEAST EXTRACT MEDIUM FOR THE ISOLATION OF FUNGI FROM SPUTUM*

DIRAN YEGIAN, D.V.M., AND JOSEPH M. KURUNG
RAY BROOK, N. Y.

SABOURAUD'S medium with a pH of 5.5 is in general use for the isolation of fungi from the sputum; however, it inhibits only to a limited degree the growth of bacteria commonly found in the sputum when incubated at a temperature of 37° C. One or more transplants may be required before the fungus is finally recovered in pure culture. The growth of the bacteria may be further inhibited by gently swabbing the surface of the medium with a tenth-normal solution of hydrochloric acid previous to the implantation of the sputum. The application of the acid to the medium does not interfere with the growth of fungi. The foregoing observation suggested the advisability of materially increasing the acidity of Sabouraud's medium for the recovery of fungi from the sputum.

A series of experiments disclosed that the optimal pH for the growth of fungi and the prevention of bacterial growth is 4.0. In order to bring Sabouraud's medium to this pH, it is necessary to modify in part the standard method of preparation as follows:

Dissolve the peptone and agar in distilled water by heating in the autoclave for fifteen minutes at 15 pounds pressure. Add a sufficient amount of an aqueous solution of 20 per cent dextrose to bring the dextrose content of the medium to 4 per cent. Under sterile conditions, and while the medium is still in the liquid state, adjust to the desired pH. The medium can be poured immediately or allowed to solidify in the container for future use.

The adjustment of Sabouraud's medium to a pH of 4.0 in the manner here described requires detailed attention and considerable time. Moreover, Sabouraud's medium is not kept in stock in many laboratories and consequently is not always available to the physician. These objections are overcome by the following simple liquid medium:

Dextrose	8.0 Gm.
Sodium chloride	1.7 Gm.
Yeast extract (Difco)	0.5 Gm.
Distilled water to make	200 c.c.
Adjust to pH 4.0	

The above medium should not be diluted when determining the pH, since it is buffered to only a slight extent if at all, and the addition of water would decrease the hydrogen-ion content.

The experiments here outlined indicate the relative value of Sabouraud's medium with pH values of 5.5 and 4.0, and dextrose-yeast-extract medium with

*From the New York State Hospital for Incipient Pulmonary Tuberculosis.
Received for publication, April 5, 1940.

a pH of 4.0 in the recovery of fungi from the sputum. In a few instances fungi present in the sputum, for example, the Penicillium, grow better at room temperature than at 37° C. It is advisable, therefore, as a routine procedure, to grow the cultures at both these temperatures.

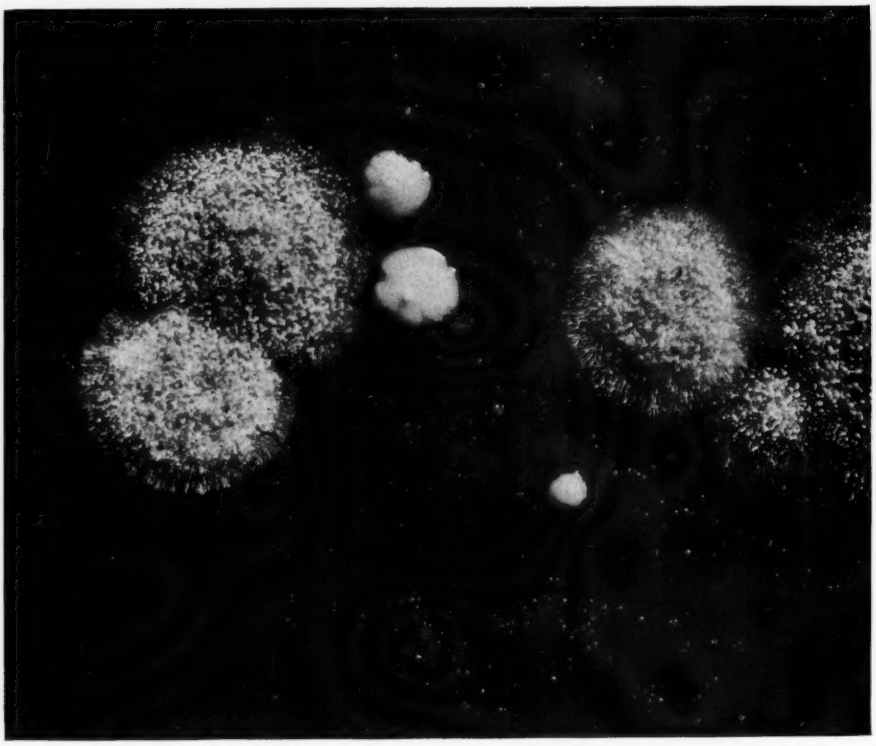
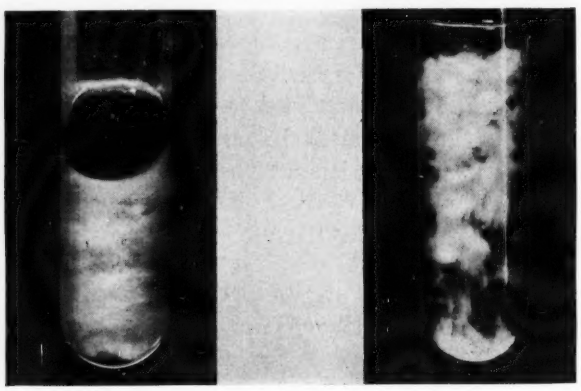


Fig. 1.—Fungus and bacterial colonies on Sabouraud's plate with pH 5.5.



A. *B.*

Fig. 2.—Pure culture in liquid media with pH 4.0. *A, Aspergillus fumigatus; B, Coccidioides immitis.*

Fifty specimens of tuberculous sputa were collected in sterile Petri dishes. A purulent portion from each specimen of sputum was implanted on these three media. The implanted medium was incubated at 37° C. and observed

daily for a period of ten days. Sixteen of the fifty specimens of sputa yielded cultures of Monilia on each of the three media. In no instance on Sabouraud's medium with a pH of 5.5 were the colonies of Monilia found in pure culture; but numerous colonies of bacteria were present on all fifty plates. The number of bacterial colonies was greatly reduced on Sabouraud's medium with a pH of 4.0, and nine of the sixteen specimens gave pure cultures of Monilia. Sputum implanted in fifty tubes of dextrose-yeast-extract medium gave no bacterial growth as determined by cultures on nutrient agar plates, and the Monilia was recovered in pure culture from sixteen tubes.

Cultures of various fungi (*Coccidioides immitis, Aspergillus fumigatus, Aspergillus niger,* Oidium, *Monilia albicans,* Geotrichum, Cryptococcus, Torula, Streptothrix, Actinomyces, and Sporotrichum) were intimately mixed with sputa from tuberculous patients and implanted on Sabouraud's medium with pH values of 5.5 and 4.0, and on dextrose-yeast-extract medium with a pH of 4.0. Colonies of each of the above-mentioned fungi were found on all three media. However, many bacterial colonies were present on all the plates on Sabouraud's medium with a pH of 5.5. The bacterial colonies were decidedly fewer in number on the Sabouraud's plates with a pH of 4.0, while on the dextrose-yeast-extract medium with a pH of 4.0, no bacterial growth was demonstrated. The above experiments indicate that the liquid dextrose-yeast-extract medium with a pH of 4.0 is superior to the solid Sabouraud's medium for the isolation of fungi from the sputum.

The dextrose-yeast-extract medium here described is composed of a few simple ingredients, is easily prepared, and affords a ready means for the recovery of fungi from the sputum.

AN EFFICIENT DISSECTING AND OPERATING TABLE*

MAX L. SWEAT, LOGAN, UTAH

THERE is need for an efficient dissecting and operating table. The one built by us has particular advantages over those now on the market. Its advantages are: (1) The mechanical construction throughout is of utmost simplicity. (2) The whole mechanism is of metal. (3) The table top can be instantly adjusted to any plane slightly short of vertical. (4) The stand may be adjusted for height. (5) The table may be used for either dissecting or operating. (6) The animal to be dissected can be rigidly fastened to the table in only a few seconds, and once in position the natural tension of its limbs prevents any loosening of the hold. (7) Both the stand and the table top can be sterilized.

When dissecting, the hooks are inserted directly into the animal's legs; and when operating, they are inserted into adhesive tape wrapped around the lower part of the legs, Several mechanical devices for holding the legs of animals without harming them were investigated, but the adhesive tape was

*From the Department of Bacteriology and Biochemistry, Utah State Agricultural College. Received for publication, April 22, 1940.

found the most practical. It neither harms the animal nor requires tedious adjusting, and is easily removed after the operation by means of ether.

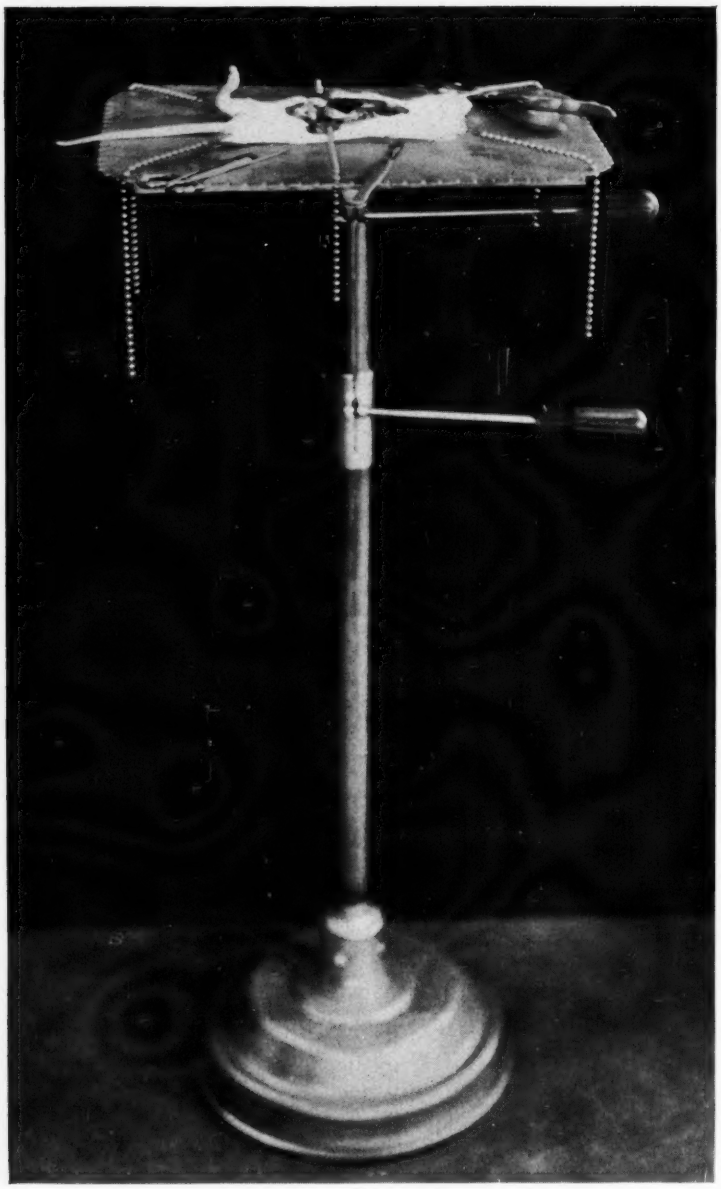


Fig. 1.

DESCRIPTION

1. *Table Top.* This is made of 18 gauge stainless steel (Fig. 1). The top is reinforced with ½ inch 90° angular strips of the same metal running around the bottom 1 inch from the edges. The size 10 by 14 inches is suitable for guinea pigs, rats, and smaller animals. Heavier gauge and larger dimensions are required for tables to accommodate large animals. Adjustments are such that

even a small mouse may be fastened to a large table, but unless work on many size animals is to be done it is advisable to make the table near the size of the animal to be used. Around the edges of the table are V-shaped grooves 3/16 inch deep and 1 inch apart. These serve to hold the bead chain in tension against the animal. Fig. 1 shows the retractors, hooks, and chains in position on the table top.

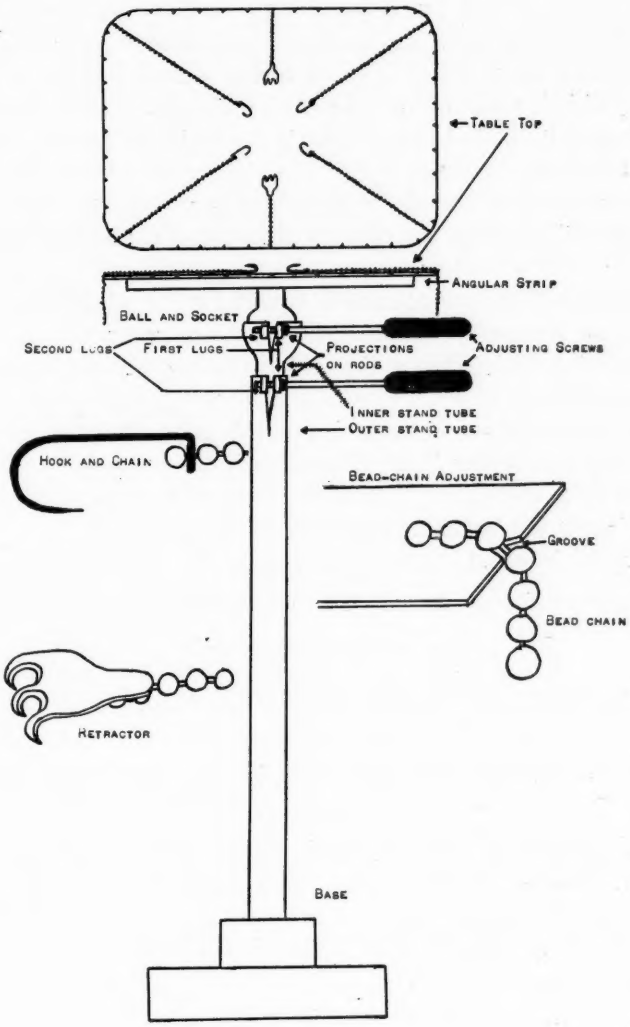


Fig. 2.

2. *Ball and Socket.* The ball is fastened to a rod welded on the bottom of the table top. The split socket is fastened to the inner stand tube. The ball and socket enables the tipping of the table in any direction. This is done by loosening or tightening the adjusting screw. A two inch ball and socket serves well for a table of this size.

3. *Adjusting Screws.* The screws are simply threaded 3/8 inch metal rods with handles. Just above the threads a projection is welded to keep the rod from going past its threads in the first lug which is slightly larger than the rod

and contains no threads. This lug is soldered on the right of either the slit of the socket or outer stand tube. The second lug, which is soldered on the left of the split, has threads of the same size as the rod. By turning the rod, the distance between the welded projection and the second lug is shortened, thus decreasing the size of the socket or outer tube as the case may be.

4. *Stand Tubes.* These are two; the inner, $1\frac{5}{16}$ inches; the outer, $1\frac{1}{2}$ inches in diameter. Both are 2 feet long. Ordinary chromium-plated brass plumbing tubing serves well. By loosening and tightening the adjusting screw, the inner tube may be raised or lowered to the desired height.

5. *Base.* This is made of two pieces of cast iron. The smaller, 2 by 4 by 4 inches, is fastened by bolts to the larger, 2 by 10 by 10 inches. The stand tube is welded to the base.

6. *Hooks.* These are made from ordinary No. 6 bait fishhooks by filing off the prongs and bending the eyes to right angles, and are then fastened to the chains.

7. *Retractors.* They may be made from 22 gauge stainless steel. They are used in the same manner as ordinary dissecting retractors.

8. *Bead chain.* This is the ordinary bead or ball chain used on light strings, sink stoppers, and key chains.

It will be noticed there is no device on this apparatus for holding the heads of animals. The hooks may be used for this if the animal is to be dissected; if to be operated on, a wide piece of adhesive tape with a hole corresponding to the point of incision in the animal's head may be wrapped entirely around the head and hooks may be inserted in the tape.

I wish to acknowledge the helpful advice and friendly criticism of Dr. J. E. Greaves.

SOME IMPROVED METHODS FOR STAINING VAGINAL SMEARS*

GEORGE N. PAPANICOLAOU, M.D., PH.D., NEW YORK, N. Y.

ABOUT three years ago Mrs. Dorothy Fuller, who was at that time working with Dr. Florence Sabin at the Rockefeller Institute, gave me a series of vaginal smears for examination. These smears were stained by Mrs. Fuller with one of Foot's modifications of Masson's trichrome method.[1] This method was briefly as follows:

Iron alum solution in oven 45° to 50° C. five minutes. Wash. Hematoxylin in oven five minutes. Picric acid five to ten minutes. Wash. Ponceau de xylidine-acid fuchsin five minutes. (Fuller used a solution of equal parts of ponceau de xylidine-acid fuchsin and 1 per cent acetic acid.) Rinse in distilled water. Phosphomolybdic acid five minutes. Rinse in distilled water. Light green five minutes. Rinse in distilled water. Acetic acid two minutes. Rinse in tap water, alcohols, and xylol. Mount in balsam.

Smears stained by this method showed a very good differentiation of the cornified cells. A double coloration of these cells was obtained; some cells

*From the Department of Anatomy, Cornell University Medical College, New York.

This study has been aided by the Committee for Research on Sex Problems of the National Research Council.

Received for publication, October 11, 1940.

were pink, some were orange, and others took on intermediate shades. Further experimentation was, however, necessary for evaluating the meaning and the importance of this differential staining of the cornified cells.

Unfortunately, the foregoing method was too long and complicated to be adopted as a standard method for staining vaginal smears. Another disadvantage was its failure to secure a differential staining of the mucous cells, which are characteristic of the early estrous stage in some lower mammals, more particularly in the guinea pig. This disadvantage would not, however, apply to human vaginal smears or to smears of such animals in which the mucous cell type is absent.

The eosin-water blue staining method which I suggested in 1933[2] permits a good differentiation between the basal cells which are stained blue, the mucous cells which are stained purple, and the cornified cells which are stained with various shades of eosin red. This method consists in treating vaginal smears, previously stained in hematoxylin, with a 0.5 per cent aqueous solution of eosin (Grübler) for four minutes and then, after rinsing in water, in a 0.5 per cent aqueous solution of water blue (Grübler) for about one minute. Shortening of the treatment with water blue would tend to intensify the staining of the cornified elements.

This staining method, though relatively short and simple, could be further simplified through a combination of eosin and water blue. The following mixture gives satisfactory results*: 1.25 Gm. of eosin (Grübler), 0.5 Gm. of water blue (Grübler), 2 Gm. of phosphomolybdic acid, and 350 c.c. of distilled water. An increase in the amount of eosin would result in sharper staining of the cornified cells.†

Since it was found that a satisfactory combination of eosin and water blue could be achieved through the acidification of the mixture, an increase in the color range of the cornified cells was attempted by the addition of orange G, acid fuchsin, and some other stains. By modifying the percentages of the various stains and by adding the proper amount of acid, various shades of orange, pink, and red were obtained. Aniline blue, light green, and other stains were substituted for water blue. The coloration of the noncornified cells could thus be changed.

After long experimentation with over 400 combinations of various imported and domestic stains, a number of satisfactory preparations were developed, some of which are described here. These preparations offer an advantage in that they can be modified to some extent for the purpose of obtaining suitable differentiation of one or another cell type.

While this work was in progress, Shorr[3, 4] described two staining methods for vaginal smears, based on a more recent modification of the Masson trichrome method by Foot.[5] The two methods suggested by Shorr are very satisfactory for human smears and are considerably shorter than the method

*This stain is also satisfactory as a general tissue stain.

†A corresponding mixture consisting of domestic stains (National Aniline and Chemical Co.) can be prepared as follows: Aniline blue, water soluble, 60 c.c. of a 0.5 per cent aqueous solution; yellow eosin, 300 c.c. of a 0.5 per cent aqueous solution; phosphomolybdic acid, 1.65 Gm. For intensifying the staining of the cornified cells, the relative amount of eosin should be raised.

used by Fuller. The second of the two methods[4] takes about six minutes after staining with hematoxylin, but it requires the following steps: (1) one minute in 1 per cent Biebrich scarlet and 0.4 per cent orange G; (2) rinsing in water; (3) one minute in equal parts of phosphotungstic and phosphomolybdic acids; (4) rinsing in water; (5) two minutes in 0.25 per cent fast green FCF; (6) one minute in 1 per cent acetic acid. With the counterstains described herein the staining takes about two minutes and consists of only one step.

A. Solutions made of partly imported and partly domestic stains.

I. Stain DA 15.

Light green F S	Grübler	0.5% Solution	20 c.c.
Orange G	Grübler	0.5% Solution	20 c.c.
Acid fuchsin	Grübler	0.5% Solution	20 c.c.
Eosin yellowish	National Aniline and Chemical Co.	0.5% Solution	40 c.c.
Acid phosphomolybdic	Merck		0.45 Gm.

II. Stain CY 18.

Wasserblau 6B extra	Grübler	0.5% Solution	10 c.c.
Orange G	Grübler	0.5% Solution	25 c.c.
Acid fuchsin	Grübler	0.5% Solution	25 c.c.
Eosin yellowish	National Aniline and Chemical Co.	0.5% Solution	60 c.c.
Acid phosphomolybdic	Merck		0.75 Gm.

III. Stain DF 11.

Aniline blue, water soluble	Grübler	0.5% Solution	12 c.c.
Orange G	Grübler	0.5% Solution	25 c.c.
Acid fuchsin	Grübler	0.5% Solution	25 c.c.
Eosin yellowish	National Aniline and Chemical Co.	0.5% Solution	60 c.c.
Acid phosphomolybdic	Merck		0.6 Gm.

B. Solutions made of domestic stains.

IV. Stain DA 19.

Light green SF yellowish	National Aniline and Chemical Co.	0.5% Solution	12 c.c.
Orange G	National Aniline and Chemical Co.	0.5% Solution	24 c.c.
Acid fuchsin	National Aniline and Chemical Co.	0.5% Solution	20 c.c.
Eosin yellowish	National Aniline and Chemical Co.	0.5% Solution	40 c.c.
Acid phosphomolybdic	Merck		0.45 Gm.

V. Stain DF 20.

Aniline blue, water soluble	National Aniline and Chemical Co.	0.5% Solution	16.5 c.c.
Orange G	National Aniline and Chemical Co.	0.5% Solution	25 c.c.
Acid fuchsin	National Aniline and Chemical Co.	0.5% Solution	25 c.c.
Eosin yellowish	National Aniline and Chemical Co.	0.5% Solution	60 c.c.
Acid phosphomolybdic	Merck		0.7 Gm.

VI. Stain DF 32.

Aniline blue, water soluble	National Aniline and Chemical Co.	0.5% Solution	12 c.c.
Orange G	National Aniline and Chemical Co.	0.5% Solution	25 c.c.
Acid fuchsin	National Aniline and Chemical Co.	0.5% Solution	21 c.c.
Eosin yellowish	National Aniline and Chemical Co.	0.5% Solution	42 c.c.
Phosphotungstic acid	Merck		0.1125 Gm.
Phosphomolybdic acid	Merck		0.225 Gm.

The solutions I to VI are more appropriate for staining human vaginal smears. For lower mammals or rodents, an increase in the percentage of the blue or green basophilic stain secures a better differentiation of the basal cells.

The complete staining procedure is as follows:

1. Smears are fixed immediately (before drying) in equal parts of 95 per cent alcohol and ether* (original method of Stockard and Papanicolaou[6]). Rinse in 70 per cent and 50 per cent alcohols and in distilled water.

2. Ehrlich's hematoxylin, one to two minutes (other hematoxylin preparations may be used).† Rinse in distilled water. Rinse a few times in one per cent hydrochloric acid (may be omitted). Keep in running water about five minutes or in lithium carbonate (3 drops of a concentrated aqueous solution in 100 c.c.) for one minute. Subsequent staining may be affected if slides are left too long in running water.

3. Rinse in distilled water. Stain in one of the counterstains (I to VI) for two minutes. (Staining for five minutes or even longer is advised when smears are thick.) Rinse in water.

4. Rinse in dioxane ten to fifteen times until smears are clear. Rinse in absolute alcohol and xylol. Mount in clarite, Canada balsam, or gum dammar.

The use of dioxane instead of ascending alcohols for dehydration shortens the staining process and improves the differential staining in human smears. It is less desirable for smears of rodents, since it modifies the staining reaction of the mucous cells.

The staining time may be considerably shortened by the omission of the hematoxylin. Slides are stained as follows:

1. Fix smears in alcohol-ether solution for from one to two minutes. Rinse well in 70 per cent and 50 per cent alcohols, and in distilled water.

2. Stain in DF 50‡ or in one of the other counterstains (I to VI) for two to three minutes. (Staining for five minutes or even longer is advised when smears are thick.) Rinse in water.

3. Rinse in dioxane until clear, then in absolute alcohol, xylol, and clarite.

Nuclei are stained red, erythrocytes orange, cornified cells red, pink, or orange, and basophile cells green or blue. The nuclei are not as dark and the cell outlines are not as sharp as with hematoxylin, but the cornified cells are more prominent and the basal cells are more transparent.

When a prompt examination of a smear is desired for diagnostic purposes this short method is of particular advantage, for it requires only about three

*Good fixation may be obtained within one to two minutes, though a fixation of from five to fifteen minutes, depending on the thickness of the smear, is more desirable. Slides may be kept in the fixative for several hours or even days without being harmed. Fixation for more than a week or two may, however, change the staining reactions of some of the cells.

†Harris hematoxylin prepared with domestic hematoxylin and ammonium alum gives very good results.

‡VII. Stain DF 50 (particularly adapted to staining without hematoxylin).

Aniline blue, water soluble	National Aniline and Chemical Co.	0.5% Solution	16 c.c.
Orange G	National Aniline and Chemical Co.	0.5% Solution	17 c.c.
Acid fuchsin	National Aniline and Chemical Co.	0.5% Solution	23 c.c.
Eosin yellowish	National Aniline and Chemical Co.	0.5% Solution	44 c.c.
Phosphomolybdic acid	Merck		0.2 Gm.
Phosphotungstic acid	Merck		0.2 Gm.

to four minutes. It was developed in cooperation with Dr. Ralph C. Benson, of the Department of Gynecology, Cornell Medical College, who finds it especially suitable for preliminary examinations at the Woman's Clinic of the New York Hospital.

The method can be further simplified by the use of dropping bottles instead of staining jars. The stain is poured over the smear, as in bacteriologic staining. The use of dropping bottles prevents evaporation or dilution of the stain and makes its periodic replacement unnecessary. In washing the slides, fluids should be poured on the reverse side so as not to wash off the smear.

The differential staining of the cornified cells is of value in interpreting human vaginal smears in both normal and pathologic cases. Totally cornified cells, like the so-called eosinophilic cells of rodents, show a strong affinity to the orange color. The term "eosinophilic" thus appears to be inappropriate for specifying these cells. The term "acidophilic" or "orangeophilic" would be more accurate. Cells of this type are found in the human vagina more frequently during premenstruum or during pregnancy.[2]

In the normal cycle the orange shades tend to predominate during the second half of the cycle. Some local irritations or infections, abortions, and certain pathologic conditions associated with bleeding are characterized by a prevalence of pink or red shades. The cornified cells, which usually appear in large numbers after a Trichomonas infection, show a preference for orange shades. A similar preference is shown by the cornified cells which follow a prolonged estrogenic treatment. Drying of the cells modifies their staining reactions. For this reason, dry smears cannot be satisfactorily stained with the afore-mentioned methods. Most dry cells take an orange or yellowish color. This may often be a source of error in evaluating smears which were partially dried up prior to fixation.

The general adoption of a standard staining method for vaginal smears was advocated by Schneider.[7] This would undoubtedly result in a more uniform description of vaginal smear findings by various workers in the field. It would be, however, difficult to reach a general agreement on the superiority of any one of the staining methods which have been thus far proposed. Besides, each one of these methods will probably offer some specific advantage over the others. The DF 50 stain without hematoxylin may be recommended for its shortness, but it does not stain nuclei as well as hematoxylin. This, though a disadvantage, may be of real advantage in the study of certain smear types. It keeps the leucocytes in the background and makes the epithelial cells stand out more distinctly. It may be used profitably in the evaluation of estrogenic or other endocrine treatments, and of cellular changes occurring during the various phases of the sex cycle. For gynecologic diagnostic work* other stains, like DF 32, DF 20, or CY 18 after hematoxylin, would be preferable, since they would bring out more cytologic details.

*The usefulness of the vaginal smear method in gynecologic diagnosis is now being investigated in cooperation with Dr. Herbert F. Traut of the Department of Gynecology.

REFERENCES

1. Foot, N. C.: The Masson Trichrome Staining Methods in Routine Laboratory Use, Stain Technol. **8**: 101, 1933.
2. Papanicolaou, G. N.: The Sexual Cycle in the Human Female as Revealed by Vaginal Smears, Am. J. Anat. **52**: 519, 1933.
3. Shorr, E.: A New Technic for Staining Vaginal Smears, Science **91**: 321, 1940.
4. Shorr, E.: A New Technic for Staining Vaginal Smears. II. Science **91**: 579, 1940.
5. Foot, N. C.: Useful Methods for the Routine Examination of Brain Tumors, Am. J. Path. **14**: 245, 1938.
6. Stockard, C. R., and Papanicolaou, G. N.: The Existence of a Typical Oestrous Cycle in the Guinea Pig With a Study of Its Histological and Physiological Changes, Am. J. Anat. **22**: 225, 1917.
7. Fletcher, P. F.: A Study of the Possible Significance of the Vaginal Smear as an Additional Factor in the Diagnosis of Incomplete Abortion—Discussion by Schneider, P. F., Am. J. Obst. & Gynec. **39**: 562, 1940.

CHEMICAL

THE SOLUTION AND DIAZOTIZATION OF BILIRUBIN IN CHLOROFORM AND OTHER ORGANIC SOLVENTS*

ROGER S. HUBBARD, PH.D., AND NORMAN HEILBRUN, M.D., BUFFALO, N. Y.

AS PART of a study of bilirubin extracted from serum by chloroform, as described in a recent note,[1] an attempt was made to study the diazo reaction of the bilirubin in such extracts. Difficulty was encountered in obtaining colors comparable with those of the various permanent standards which have been recommended from time to time,[2-4] even when various buffer systems to insure the correct hydrogen-ion concentration were used. Attention was, therefore, turned to the use of standards prepared from bilirubin dissolved in chloroform, as originally recommended by van den Bergh.[2] The bilirubin used in these experiments† was not easily completely soluble in chloroform. Without the aid of heat, the strongest solutions that could be prepared contained about 10 mg. of bilirubin per 100 c.c. Solutions with concentrations of this magnitude were used by van den Bergh in standardizing the method when it was first described[2] and have been more recently employed by Malloy and Evelyn[5] in a photoelectric modification of the technique. By the use of heat, solutions containing 40 to 50 mg. per 100 c.c. could be prepared.‡ The long period of heating sometimes found necessary seemed inadvisable when working with a compound such as bilirubin, and we wished, if possible, to prepare stock solutions containing even higher concentrations of bilirubin than this. Bilirubin in alkaline solvents, both inorganic and organic, were quite unstable. It was finally found that if phenol were added to various organic solvents, very strong solutions of bilirubin could be prepared, and that the stability of such solutions in diffused light was much greater than in the pure solvents. This increased solubility and stability were demonstrated in solutions in chloroform, xylene, acetone, and methyl and ethyl alcohol.

Most of the experimental work was done upon bilirubin dissolved in a mixture of chloroform and phenol, made by dissolving 10 Gm. of phenol in 100 c.c. of chloroform. This solution, prepared freshly before use, would dis-

*From the Buffalo General Hospital and the University of Buffalo Medical School.

This work was aided by a grant from the Junior Board of the Buffalo General Hospital.

Received for publication, November 1, 1940.

†For the greater part of this work a preparation of bilirubin obtained from the Eastman Kodak Co., was used.

‡In a recent technical bulletin[6] the use of a stock chloroform solution of bilirubin containing 50 mg. of bilirubin in 100 c.c. is recommended. No directions for preparing this solution are given. The reference furnished is to a paper of Malloy and Evelyn.[5] In the original article the standard used contained 10 mg. of bilirubin dissolved in 100 c.c. of chloroform. We have not found any other reference to the use of chloroform solutions of bilirubin of this magnitude.

solve the bilirubin used to give concentrations as high as 400 mg. per 100 c.c. without the aid of heat; for the most part the stock solution used contained 250 mg. per 100 c.c.

The problem of diazotizing such solutions was next investigated. In the first experiments the chloroform solution was diluted with methyl alcohol, ethyl alcohol, or acetone until a homogeneous mixture with the water diazo reagent of Thannhauser and Andersen[7] was obtained. When high concentrations of bilirubin were present, this method was satisfactory, but the amounts contained in chloroform extracts of serum gave colors which were so faint that quantitative measurement of them was difficult or impossible. Various attempts to obtain suitable colors with diazo reagents soluble in ethyl alcohol were unsuccessful, but finally a fairly satisfactory reagent was found. This solution was prepared as follows: 0.1 Gm. of sulfanilic acid was dissolved in 2 c.c. of concentrated hydrochloric acid and 4 c.c. of water. To obtain a satisfactory solution the mixture was heated upon a boiling water bath, and the small lumps of the acid were mashed with a glass rod. The concentrated acid solution was cooled and made up to 100 c.c. with absolute methyl alcohol. The preparation is a saturated solution of sulfanilic acid; some crystals separate on standing. The solution corresponds to solution A of van den Bergh. Solution B was prepared by dissolving 0.5 Gm. of sodium nitrite in 100 c.c. of methyl alcohol. Fifteen minutes before use 0.3 c.c. of solution B was added to 10 c.c. of solution A. Immediately before use one volume of the mixture was diluted with an equal volume of absolute methyl alcohol. The amount of water present in this solution is only slightly less than that which will cause a separation of water when added to chloroform, xylene, or benzene, and this renders the use of absolute alcohol in preparing the reagent necessary.

The diazo reaction was studied upon dilutions of the strong bilirubin solution already described. These dilutions were made with pure chloroform. The technique used for developing the color was as follows: To 2 c.c. of the chloroform solutions 1 c.c. of the diluted mixed diazo reagent was added and the solutions were mixed. After fifteen minutes the colors were read in a microcolorimeter. To ensure the presence of a maximum degree of acidity, and so uniformity of color, 1 c.c. of a strong (80 Gm. to 100 c.c.) solution of trichloracetic acid in methyl alcohol was usually added just before the reading was made. As far as could be determined, however, this precaution was not necessary in the bilirubin solutions used as standards or in the chloroform blood extracts, for soluble buffer compounds were not present in concentrations great enough to affect the color. If fairly large amounts of a suitable buffer, such as ammonium acetate, were present, the color, which was normally a purple, had a marked reddish tint, and this source of error could be avoided or greatly lessened by the addition of trichloracetic acid after diazotization.

The method could be successfully applied to solutions containing from 25 to 0.1 mg. of bilirubin per 100 c.c. of chloroform, i.e., in dilutions of the stock solution ranging from 1:10 to 1:300. When higher concentrations were present, the color obtained was too dark to be read in the colorimeter, and weaker solutions gave colors which were too faint to be measured accurately. Within the

range given the relationship between the color obtained and the amount of bilirubin present was linear, i.e., each solution read correctly when matched against those containing twice or one-half as much bilirubin.

Since the stock solution contained phenol, it was necessary to investigate the development of color when phenol solutions in chloroform were diazotized by the technique described. When such solutions contained 10 per cent of phenol, a greenish tint developed slowly. When 5 per cent or less phenol was present, no color could be observed when the method was carried out, nor could any measurable effect upon the weakest solutions of bilirubin studied be distinguished. Since the maximum phenol concentration in any of the solutions used was 1 per cent, there could have been no significant effect of phenol upon the results reported.

Although the actual amounts of phenol in the diazotized mixtures could not have measurably affected the colors obtained, it seemed possible that some reaction might occur between phenol and the bilirubin in the stock solution which would affect the color obtained upon diazotization. To exclude such a source of error, chloroform dilutions of the stock solution were made both soon after it had been freshly prepared and after it had been kept for months in the icebox. These were diazotized simultaneously with solutions of bilirubin prepared in various ways. These control standards included very dilute solutions of bilirubin in pure cold chloroform, stronger chloroform standards prepared by the aid of heat, and solutions prepared by dissolving bilirubin in water containing minimum amounts of alkali. In the last instance the diazo procedure described above could not be used. These solutions were diluted with alcohol, and the chloroform-phenol solution was diluted with alcohol until the solution was miscible with water and the water-soluble diazo reagent of Thannhauser and Andersen could be used. Precautions were taken to insure the presence of equal concentrations of water, alcohol, and chloroform in the dilute solutions finally studied. No qualitative or quantitative differences in the color given by the reaction could be detected when bilirubin solutions prepared from the stock containing phenol were compared with bilirubin prepared in these three ways.

It seemed desirable to determine whether the colors given by different bilirubin preparations on diazotization were strictly comparable with each other. Many different lots of bilirubin obtained from the Eastman Kodak Co. gave identical results when treated by the technique outlined. A specimen of bilirubin from the Wilson Co., which had a somewhat different consistency from that used in most of the study, and one imported from Germany marked "Bilirubin Homberg," manufactured by Chemische Pharmazeutische A. G. Bad Homburg according to the directions of von Bergman and Eilboth, also gave colors which were the same qualitatively and quantitatively as those obtained from the bilirubin purchased from the Eastman Kodak Co.

The length of time during which the standard would not deteriorate under ordinary working conditions was investigated. The stock solution decomposed rapidly in direct sunlight, but even in clear glass the solution kept its strength for about six months when exposed daily for two or three hours to diffused daylight and artificial light in the laboratory if placed in a cupboard or icebox

when not in use. A slight darkening of the initial light yellow tint took place, but the intensity of the color obtained upon treatment with diazo reagents remained unaltered. This finding was confirmed repeatedly with stock solutions of various concentrations. The test used was to compare the color obtained from the old bilirubin standard with that given by one weighed and dissolved immediately before use.

Besides these experiments with chloroform solutions of bilirubin, the diazo reaction described was carried out in various other solvents. In aqueous preparations the method was not satisfactory, but in various organic solvents only minor difficulties were encountered. When the stock solution of bilirubin in chloroform and phenol was diluted with benzene, xylene, acetone, ether, or carbon tetrachloride, the results were practically identical with those obtained when chloroform was used. The colors were not qualitatively the same in all the solvents, but in each solvent the colors were alike and were strictly proportional to the amount of bilirubin present. The lowest concentration which gave a visible color varied somewhat in the different experiments. With acetone, for example, the color given by a concentration of 0.04 mg. per 100 c.c. could just be distinguished, while benzene gave a slight color in solutions one-half as concentrated. All the solvents were fairly satisfactory when the concentration of bilirubin was 25 mg. per 100 c.c., although in some instances, notably benzene and carbon tetrachloride, the color given by such concentrations was very intense.

Results with absolute ethyl, methyl and butyl alcohol were less satisfactory. Precipitation took place in the higher concentrations with the first two solvents named, and in the butyl alcohol solution the bilirubin showed a marked tendency to separate on the glass of the container. In the range in which bilirubin was soluble the diazotization was satisfactory. The lowest visible color when dilutions were made with butyl alcohol was 0.08 mg. per 100 c.c. The methyl alcohol diazo reagent possessed no advantage over reagents prepared in water when the bilirubin was dissolved in solvents easily miscible with water.

Certain experiments were carried out on the diazotization of bilirubin extracted by chloroform. Bilirubin was dissolved in water containing a minimum amount of alkali and the pigment was extracted with chloroform after acidifying with hydrochloric and acetic acids. When an aliquot of such extracts was diazotized as described, the recovery ranged from 95 to 100 per cent of the theoretical values. Solutions prepared by diluting the stock solution in chloroform and phenol were used as standards in these experiments.

Freshly prepared solutions of bilirubin in alkali were added to serum. After acidifying and extracting with chloroform, the extract was diazotized as described. The recovery ranged from 90 to 95 per cent of the amount added. When normal human serum was acidified and extracted with chloroform, the amount recovered gave colors in some instances which corresponded approximately with those expected if the serum had contained 0.05 mg. of bilirubin per 100 c.c. Approximate measurements of the yellow color, of such an extract by the method of Soffer[8] indicated the presence of a higher concentration of bilirubin than did the diazo color. The results suggested that yellow

pigments which were soluble in chloroform and were not diazotized were present in serum. No indication of the presence of a chloroform-soluble diazotizable compound other than bilirubin was obtained. When serum from patients with a marked degree of jaundice was extracted with chloroform, identical results were obtained, within the limits of accuracy of the methods, by the diazo technique and by measurement of the yellow color.

SUMMARY

Solutions containing as much as 400 mg. of bilirubin per 100 c.c. can be prepared by dissolving the pigment in a solution of chlorofrom containing 10 per cent phenol. Solutions of bilirubin in organic solvents containing phenol are more stable than in pure organic solvents. Phenol, unless present in high concentrations, has no effect upon the color given by bilirubin treated with diazo reagents. Diazotization of bilirubin dissolved in various organic solvents can be successfully accomplished by the use of a diazotizing solution prepared in methyl alcohol.

REFERENCES

1. Heilbrun, N., and Hubbard, R. S.: The Measurement of the Chloroform-Soluble Fraction of Bilirubin in Persons With Jaundice and Its Significance, J. LAB. & CLIN. MED. **26**: 576, 1940.
2. Bergh, A. A. Hymans van den, and Snapper, J. D.: Die Farbstoffe des Blutserums, Deutsches Arch. f. klin. Med. **110**: 540, 1913.
3. McNee, J. W., and Keefer, C. S.: The Clinical Value of the van den Bergh Reaction for Bilirubin in Blood, With Notes on Improvements in Its Technique, Brit. M. J. **2**: 52, 1925.
4. Rhamy, B. W., and Adams, P. H.: A New Standard for the van den Bergh Test, J. LAB. & CLIN. MED. **13**: 87, 1927.
5. Malloy, H. T., and Evelyn, K. A.: The Determination of Bilirubin With the Photoelectric Colorimeter, J. Biol. Chem. **119**: 481, 1937.
6. Klett Summerson Photoelectric Colorimeter, General directions, Detailed analytical procedures, Klett Manufacturing Co., New York.
7. Thannhauser, S. J., and Andersen, E. D.: Methodik der quantitativen bilirubinbestimmung im menschlichen serum. Über die Ehrlich-Proschersche Reaktion, Deutsches Arch. f. klin. Med. **137**: 179, 1921.
8. Soffer, L. J.: Bilirubin Excretion as a Test for Liver Function During Pregnancy, Bull. Johns Hopkins Hosp. **52**: 365, 1933.

THE FORMOL-GEL REACTION*

Description of a Modified Method for Quantitative Estimation of Serum Globulin in Hyperglobulinemia and a Critical Analysis of the Reaction

Fernando Biguria, M.D., and Sawyer Foster, M.D.
Boston, Mass.

INTRODUCTION

THE formol-gel reaction, first described by Gaté and Papacostas,[1] is a test for hyperglobulinemia regardless of the cause. It is performed by adding 2 drops of approximately 40 per cent formaldehyde solution to 1.0 c.c. of blood serum. Either solid gelification (Gaté-Papacostas test), or an increase in opacity (Napier's test)[2-7] occurs in a "positive" reaction after a period of twenty-four hours' standing at room temperature. For a thorough treatment of the development of this test and its modifications, the work of Bing[8] is recommended.

Wise and Gutman[9] studied the formol-gel reaction in 113 sera with simultaneous chemical determinations of the several protein fractions. According to their criteria, a test was considered positive when it showed either an *increase in viscosity* from 1 + to 4+, as measured by a change from oily flow to a solid gel, or an *increase in opacity* from 1+ to 4,+ as measured by a change from faint haziness to complete opacity. Almost invariably a positive reaction occurred when the globulin level was 4.0 per cent or above, and a negative reaction occurred at globulin levels below 3.4 per cent. Sera containing 3.4 to 4.0 per cent globulin, however, did not give a positive test consistently.

De Vries[10] disregarded the opacity type of reaction and studied 28 sera which showed solid gelification. He concluded that the reaction was usually negative when the globulin level was below 3.6 per cent, and positive when above 3.7 per cent. He also noted that when sera were diluted with isotonic saline below globulin levels of 3.6 per cent gelification no longer took place.

The role of albumin in the formol-gel reaction has been discussed by various workers,[8-10] the consensus of opinion being that albumin plays a minimal part, if any, in the reaction. The most conclusive studies are those of Napier,[3] who showed by precipitation reaction and dialysis that the formol-gel test is specific for globulin, and that albumin has no influence.

In the present study the viscosity of formaldehyde-treated sera was measured and correlated with gelification (maximum viscosity change) and with the concentration of serum globulin. A modification of the formol-gel reaction is described for the quantitative estimation of serum globulin in hyperglobulinemia. In addition, observations were made on the relation of opacity to the content of serum globulin.

*From the First and Third (Tufts) Medical Services, Boston City Hospital, and the Department of Medicine, Tufts College Medical School.
Aided by a grant from the Charlton Research Fund, Tufts College Medical School.
Received for publication, August 26, 1940.

METHODS

Serum was obtained from samples of venous blood allowed to clot, the samples being taken during the fasting state. The albumin-globulin separation was performed by a modification of Howe's method,[11] employing 22.5 per cent sodium sulfate at 37.5° C. All nitrogen determinations were made by the micro-Kjeldahl method.[12]

Samples of serum containing an elevated concentration of globulin were diluted progressively with saline and allowed to stand at room temperature for twenty-four hours. These mixtures showed progressive decrease in the apparent viscosity from gelification to unaltered viscosity. At one dilution of the formaldehyde-treated serum the air bubbles introduced by shaking the tube did not rise to the surface but remained stationary. Since this phenomenon appeared consistently and was readily detected, it was arbitrarily chosen as the end point (*"stationary bubble" end point*). Repeated observations revealed that the concentration of serum globulin which correlated with this end point was 3.1 Gm. per cent. It was also noted that when air bubbles rose very slowly in a mixture, the globulin level was between 2.9 and 3.1 Gm. per cent.

The *modified formol-gel reaction* was performed by introducing serum into test tubes of 8 mm. bore in the following amounts: 1.0 c.c., 0.96 c.c., 0.92 c.c., etc., diminishing the quantity of serum in each subsequent tube by 0.04 c.c. Enough saline was added to each tube to make the contents up to 1.0 c.c. After mixing, 2 drops of approximately 40 per cent formaldehyde solution (U.S.P.) were added. The tubes were tightly stoppered, shaken, and allowed to stand at laboratory temperature for twenty-four hours. The tube demonstrating the above-mentioned "stationary bubble" end point was then selected, and the globulin content, in grams per 100 c.c. of serum, was calculated by the following formula:

$$\text{Grams per cent globulin} = \frac{3.1}{y}$$

where y equals the fraction of 1.0 c.c. of serum present in the tube showing the end point.

Example: If the "stationary bubble" end point occurred in the tube containing 0.80 c.c. of serum, the calculated globulin concentration $= \frac{3.1}{0.80} = 3.87$ Gm. per cent.

The opacity of formaldehyde-treated sera was compared after twenty-four hours with corresponding untreated samples. The degree of opacity change was read according to the criteria of Wise and Gutnam.[9]

Direct observations of the viscosity (relative to water = 1) of fresh horse sera, treated as in the modified formol-gel reaction, were made by measuring the rate of flow in an Ostwald pipette[13] at a temperature of 25° C. Similar observations were made also on corresponding dilutions of untreated sera.

RESULTS

1. *Direct Viscosity Studies on Horse Sera.*—Fig. 1 represents the direct measurements of viscosity made on three horse sera.* The apparent globulin

*Horse serum was used because of its high concentration of globulin, and because large amounts of serum are required for the Ostwald pipette.

content of the various mixtures was calculated from the formula using the "stationary bubble" end point. The mixture at this end point was too viscid to flow through the Ostwald pipette. The highest concentration of globulin in which the viscosity could be measured corresponded to a calculated value of 2.85 Gm. per cent. Analysis of the viscosity curve of the formaldehyde-treated sera shows that as the globulin content increased from 0.0 to 2.5 Gm. per cent, the viscosity rose slowly from 1.0 to 6.5; as the globulin content increased from 2.5 to 2.85 Gm. per cent, there was a remarkable rise in viscosity from 6.5 to 21.8. On the other hand, analysis of the viscosity of corresponding dilutions of untreated sera showed that as the globulin increased from 0.0 to 2.85 Gm. per cent, the viscosity rose only from 1.0 to 1.7.

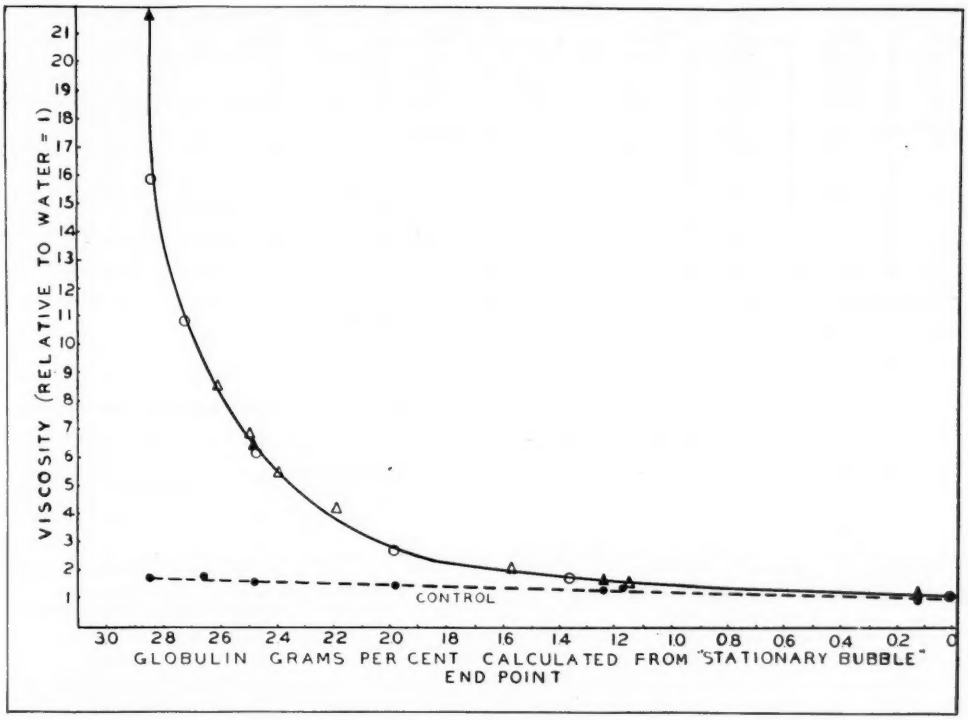


Fig. 1.—Direct measurements of viscosity on three horse sera twenty-four hours after the addition of formaldehyde.

The "stationary bubble" end point apparently corresponded to a definite but not measurable viscosity value, since diluted samples in which the viscosity could be measured followed a constant type of curve.

2. *Application of the Modified Formol-Gel Reaction.*—Eighteen human sera and one horse serum (Table I) showing the "stationary bubble" end point (or changes close to the end point) were studied using the modified formol-gel reaction. By application of the formula it was possible to calculate the apparent globulin content of 13 of these sera. In the other 6 sera in which the end point was not obtained, the globulin was estimated to be 2.9 or 3.0 Gm. per cent (see Methods). The globulin values obtained by the modified formol-gel reaction are compared with those obtained by the Howe method. The differences varied from 0.01 to 0.57 Gm. per cent, with an average of 0.22 Gm. per cent for all cases.

TABLE I

CASE NO.	HOWE METHOD-MICRO-KJELDAHL			FORMOL-GEL REACTION	DIFFERENCE IN GLOBULIN (GM. %)	DAYS BETWEEN DETERMINATIONS	CLINICAL DIAGNOSIS
	TOTAL PROTEIN (GM. %)	SERUM ALBUMIN (GM. %)	SERUM GLOBULIN (GM. %)	SERUM GLOBULIN (GM. %)			
1	5.53	2.02	3.51	3.5	−0.01		Alcoholic cirrhosis, ascites
2	8.11	4.70	3.41	3.4	−0.01		Rheumatic heart disease
3	6.98	3.49	3.49	3.7	+0.21	2	Gonococcus arthritis
3a	7.31	3.38	3.93	3.7	+0.23		
4	7.00	3.62	3.38	3.7	+0.32	4	Gonococcus arthritis
4a	7.37	3.25	4.12	3.9	−0.22		
5	5.73	1.85	3.88	3.9	+0.02		Alcoholic cirrhosis, ascites
6	5.83	2.42	3.41	3.1	−0.31	7	Carcinoma of prostate with metastasis, ascites
6a	5.50	2.39	3.11	3.0	−0.11		
7	6.54	2.57	3.97	3.9	−0.07		Hepatitis
8	7.27	4.07	3.20	2.9	−0.30	5	Catarrhal jaundice
8a	6.91	3.96	2.95	3.0	+0.05		
9	6.02	2.55	3.47	2.9	−0.57	5	Generalized anasarca, ? beriberi heart
9a	5.63	2.49	3.14	3.0	−0.14		
10	6.23	3.59	2.64	3.0	+0.36		Rheumatic heart disease, pericarditis
11	6.57	3.63	2.94	3.1	+0.16		Rheumatic heart disease, pericarditis
12	6.22	2.50	3.72	3.5	−0.22		Normal horse
13	7.33	3.98	3.35	3.9	+0.55	4	Syphilis, cirrhosis, jaundice
13a	7.40	3.92	3.48	3.7	+0.22		

a Represents determinations repeated on the same patient.

TABLE II

CASE NO.	HOWE METHOD—MICRO-KJELDAHL			FORMOL-GEL REACTION	DAYS BETWEEN DETERMINATIONS	CLINICAL DIAGNOSIS
	TOTAL PROTEIN (GM. %)	SERUM ALBUMIN (GM. %)	SERUM GLOBULIN (GM. %)	OPACITY RANGE (1+ to 4+)		
1	6.83	3.99	2.84	++		Carcinoma of breast with metastasis
2	7.53	4.66	2.87	+++		Rheumatic heart disease
3	6.31	4.01	2.29	+		Coronary thrombosis
4	6.52	3.93	2.59	+		Multiple myeloma
5	7.18	4.72	2.46	++		Coronary thrombosis
6	6.28	3.96	2.32	++	19	Diabetes mellitus
6a	6.75	4.45	2.30	+		
7	6.08	3.24	2.84	++		Normal horse used for bleeding
8	3.44	1.44	2.00	++	4	Nephrotic stage of glomerulonephritis*
8a	3.13	1.33	1.80	++		
9	4.28	1.86	2.42	++		Intercapillary glomerulonephritis*
10	6.50	5.05	1.45	+	3	Normal
10a	6.68	4.49	2.19	+		
11	6.50	4.75	1.75	+		Normal
12	6.16	4.52	1.64	+		Normal

*Opacity readings were difficult in Cases 8 and 9, due to high cholesterol values.
a Represents determinations repeated on the same patient.

In this series the modified formol-gel reaction was used for estimation of globulin in hyperglobulinemia, with an average difference of 0.22 Gm. per cent globulin compared to values obtained by the Howe method. This error is similar to that of the Howe method itself.

3. *Relation of Opacity to Hyperglobulinemia.*—Fifteen sera (Table II) were studied which did not show the "stationary bubble" end point, but did show various degrees of opacity after the usual procedure with formaldehyde. By the Howe method the globulin content of these sera ranged from normal values to moderate elevations. Each of these sera showed some degree of opacity (1+ to 3+) which apparently bore no constant relation to its globulin content.

It may, therefore, be concluded that since opacity changes in formaldehyde-treated specimens are present in sera obtained from individuals with normal globulin values, opacity cannot be regarded as a reliable index of hyperglobulinemia.

DISCUSSION

A modified formol-gel reaction is described for estimating the globulin content of sera in hyperglobulinemia. This method has a decided advantage over the standard formol-gel reaction which can only be used as a qualitative indication of hyperglobulinemia.

The "stationary bubble" end point used in this modified technique has been standardized against the Howe method for globulin determination. Howe's method, however, has definite limitations inasmuch as the usual salting-out procedures produce incomplete separation of the albumin and globulin fractions.[14, 15] The method devised by Tiselius[16] precludes this source of error. Hence, its use in the standardization of the "stationary bubble" end point should result in further accuracy of the modified formol-gel reaction.

The close relationship between the degree of viscosity and the calculated globulin content strongly suggests that viscosity measurements may be used for estimation of globulin.

We gratefully acknowledge the assistance we have received from the following members of the Thorndike Memorial Laboratory: Miss Margaret A. Adams, for the chemical studies on proteins; Miss Constance Brooks, for the viscosity experiments; and Drs. T. H. Ham, W. T. Salter, and F. H. L. Taylor, for their interest and valuable suggestions and criticisms.

REFERENCES

1. Gaté, J., and Papacostas: Une nouvelle réaction des sérums syphilitiques; formol-gelification, Compt. rend. Soc. de biol. **83**: 1432, 1920.
2. Napier, L. E.: A New Test for Kala-azar, Indian M. Gaz. **56**: 338, 1921.
3. Ibid.: A New Serum Test for Kala-azar, Indian J. M. Research **9**: 830, 1921.
4. Ibid.: Practical Experience With Aldehyde Test, Indian M. Gaz. **58**: 104, 1923.
5. Ibid.: New Serological Test for Kala-azar, Indian M. Gaz. **62**: 362, 1927.
6. Ibid.: Critical Examination of Antimony Tests for Kala-azar, Indian M. Gaz. **63**: 687, 1928.
7. Ibid.: Notes on the Significance of Antimony Test in Diagnosis of Kala-azar, Indian M. Gaz. **64**: 669, 1929.
8. Bing, J.: The Formol-Gel Reaction and Other Globulin Reactions, Acta med. Scandinav. **91**: 336, 1937.
9. Wise, C. R., and Gutman, A. B.: The Formol-gel Reaction. A Convenient Preliminary Test for Hyperglobulinemia, Am. J. M. Sc. **194**: 263, 1937.
10. de Vries, A.: On the Connection Between the Formol-gel Reaction and the Blood Proteins, Acta med. Scandinav. **99**: 425, 1939.
11. Howe, Paul E.: The Use of Sodium Sulphate as the Globulin Precipitant in the Determination of Proteins in Blood, J. Biol. Chem. **49**: 93, 1921.
12. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry. Methods. Baltimore, 1932, Williams & Wilkins Co., p. 691.
13. Findlay, A.: Physical Chemistry for Students of Medicine, London, 1927, Longmans, Green & Co., p. 27.

14. Butler, A. M., and Montgomery, H.: The Solubility of the Plasma Proteins. I. Dependence on Salt and Plasma Concentrations in Concentrated Solutions of Potassium Phosphate, J. Biol. Chem. **99**: 173, 1932.
15. Butler, A. M., Blatt, H., and Southgate, H.: The Solubility of the Plasma Proteins. II. Dependence on pH, Temperature, and Lipid Content in Concentrated Solutions of Potassium Phosphate and Application to Their Separate Precipitation, J. Biol. Chem. **109**: 755, 1935.
16. Tiselius, A.: Electrophoresis of Serum Globulin. II. Electrophoretic Analysis of Normal and Immune Sera, Biochem. J. **31**: 1464, 1937.

METHOD FOR THE QUANTITATIVE ESTIMATION OF ACETONE AND ACETOACETIC ACID IN URINE*

Preliminary Report

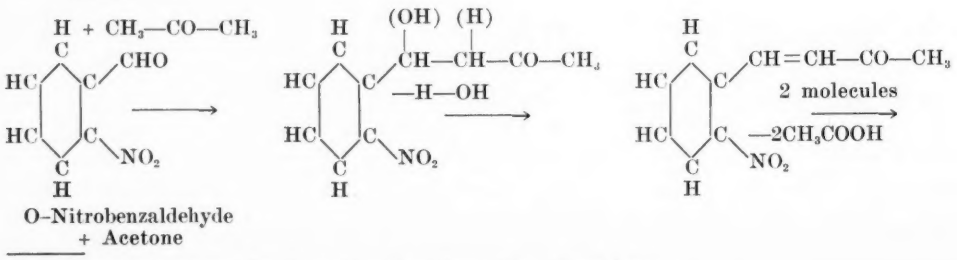
Lorraine F. Noyes, B.S., East Providence, R. I.

THOUGH a number of methods for the quantitative determination of ketone bodies have been developed,[1-7] most of them are too time-consuming and laborious to justify their routine use in the average clinical laboratory. A definite need exists for a simple quantitative method of determining acetone and acetoacetic acid with ketoses due to diet or disease. To meet partially the need for a quantitative method for measuring ketone bodies in patients receiving ketogenic diets, Osterberg and Helmholz[8] modified a qualitative method for acetone and acetoacetic acid which allows a roughly quantitative estimation of beta-hydroxybutyric acid. The present report describes a method truly quantitative in its range, requiring no distillation or time-consuming gravimetric procedure, and for which the reagents are readily available. It has been used successfully in the laboratory of the Emma Pendleton Bradley Home for over a year.

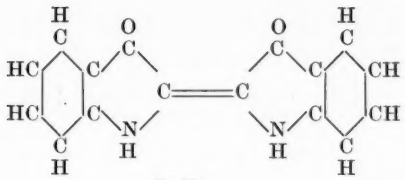
THEORETICAL BASIS

This method is based on the Baeyer and Drewsen[9] synthesis of indigo. Acetone and ortho-nitrobenzaldehyde, in the presence of sodium or potassium hydroxide, condense to give indigo. As an early means for the synthetic production of indigo this reaction was found to be nearly quantitative. By extracting the indigo produced with chloroform, the condensation may be adapted to the quantitative estimation of acetone and acetoacetic acid as acetone when these are present to a pathologic degree in urine.

Reaction:



*From the Emma Pendleton Bradley Home, East Providence.
Received for publication, August 2, 1940.



Indigo

While the reaction is quantitative when large amounts of acetone and ortho-nitrobenzaldehyde are present, it is not quantitative when only small amounts of acetone are present. However, the amount of indigo formed is proportionate to the acetone present and to the time of standing. Natural standards must be used, made up at the same time as the unknown, and from acetone solutions of low concentration. Indigo is only slightly soluble in chloroform, so that care must be taken when large amounts of indigo are formed that the dye is completely extracted. Several extractions may be necessary when acetone and acetoacetic acid are present in large quantities.

Too great an excess of ortho-nitrobenzaldehyde or alkali or both will cause secondary reactions, so that the chloroform extract will be greenish and useless for comparison. The solution of indigo in chloroform remains stable for several hours and, if the concentration of indigo is not too great, for several days. Acetaldehyde and pyruvic acid also condense with ortho-nitrobenzaldehyde to give indigo. However, acetaldehyde is never present in urine, and as the method is only useful in the pathologic range, the amount of pyruvic acid present at any time is so small that the error thus introduced is negligible. Other substances present in normal or pathologic urines have not interfered with the reaction either by reacting with the reagents or by preventing the reaction. A review of the literature and practical experience indicate that the condensation is specific for acetone, acetaldehyde, and pyruvic acid.

METHOD

Reagents.—Five per cent solution of ortho-nitrobenzaldehyde* in 95 per cent ethyl alcohol.

Ten per cent potassium hydroxide.

Solutions.—Solutions of acetone containing 1.0 and 0.5 mg. of acetone per cubic centimeter should be made up and accurately titrated with iodine. Standards for urines giving a faintly positive test for acetone and acetoacetic acid should be made from 1 or 2 c.c. of the 0.5 mg. per cubic centimeter of standard, and for those giving a strongly positive test from the 1.0 mg. per cubic centimeter of standard.

Procedure.—Twenty cubic centimeters of a urine giving a positive test for acetone or acetoacetic acid are transferred to a large test tube fitted with a delivery tube. Fresh cork stoppers or rubber stoppers covered with tin foil must be used. The delivery tube need not be cooled during the time the specimen is in the water bath. The tip of the delivery tube is placed well below the surface of 4 c.c. of distilled water in a small test tube. The sample is immersed in the boiling water bath for ten minutes to precipitate proteins and to convert the

*The ortho-nitrobenzaldehyde was secured from the Eastman Chemical Co.

acetoacetic acid to acetone. The boiling of the water bath is so adjusted that very little vapor is transferred to the receiver. The sample is then cooled to room temperature. The receiver and the delivery tube are rinsed with small portions of distilled water and the washings are added to the sample, which is then measured and filtered.

TABLE I

RECOVERY OF ACETONE AND ACETOACETIC ACID FROM URINE BY NEW METHOD

All urines are those of children between the ages of 6 and 12 years. The results are expressed as milligrams of acetone per 100 c.c. of urine

SPECIMEN	PREFORMED ACETONE AND ACETOACETIC ACID BY NEW METHOD (0.2 MG. ACETONE ADDED TO EACH SAMPLE)	PREFORMED ACETONE AND ACETOACETIC ACID BY METHOD OF BEHRE AND BENEDICT	PREFORMED ACETONE AND ACETOACETIC ACID BY NEW METHOD	ACETONE ADDED	ADDED ACETONE RECOVERED BY NEW METHOD	ADDED ACETONE RECOVERED BY METHOD OF BEHRE AND BENEDICT
Normal	1.08	1.021	--	10.00	9.48	9.72
Normal	0.38	.43	--	20.00	19.76	19.68
Normal	0.46	.58	--	40.00	40.21	39.46
Normal	1.43	1.57	--	40.00	39.93	39.71
Ketogenic urine Diet 1.5:1	--	3.59	3.74	--	--	--
Ketogenic urine Diet 2.5:1	--	13.23	13.67	--	--	--
Ketogenic urine Diet 3:1	--	40.27	41.82	--	--	--
Ketogenic urine Diet 4:1	--	113.61	118.43	--	--	--
Diabetic urine	--	23.11	22.86	10.00	9.92	9.76
Diabetic urine	--	3.29	3.16	10.00	9.67	9.72

A volume of filtrate equivalent to 10 cubic centimeters of urine is transferred to a large test tube. One cubic centimeter of the 5 per cent alcoholic solution of ortho-nitrobenzaldehyde and 2 c.c. of 10 per cent potassium hydroxide are added to the unknown. It is essential that the reagents be added in this order to prevent the precipitation of the ortho-nitrobenzaldehyde. The unknown and a standard, which is set up at the same time and with the same quantity of reagents, are allowed to stand for one hour. The indigo is then extracted with chloroform. Three cubic centimeters of chloroform are sufficient to extract the indigo when the acetone concentration is low. When much indigo is present, it may be necessary to make several extractions. A separatory funnel is used for the extraction and the extraction will be faster if warm water is added to the unknown. Where the tap water is relatively pure it may be used. The extract is transferred to a calibrated tube containing 2 c.c. of water. By the addition of chloroform, the standard and the unknown are adjusted to approximately the same indigo concentration. Cloudiness of the chloroform extract, often present after standing, may be removed by warming the solution under tap water. Indigo is calculated as acetone per cubic centimeter of chloroform in the standard. Standard and unknown are then compared in the colorimeter.

$$\frac{RS}{RU} \times A = \frac{B \times C \times V}{v} =$$ milligrams of acetone plus acetoacetic acid as acetone in the total specimen.

RS is the reading of the standard, usually 20; RU is the reading of the unknown; A is the milligrams of acetone per cubic centimeter of chloroform of the standard; B is the milligrams of acetone per cubic centimeter of chloroform of the unknown; C is the number of cubic centimeters of chloroform of the unknown; V equals the total volume of the specimen; v equals the volume of the sample.

DISCUSSION

In the upper range of total ketone bodies (over 0.2 per cent) the method was found to be truly quantitative. In the lower range the results were low. It seems probable on the basis of further studies now in progress that the range of this method may be increased and that it may be modified to measure total ketone bodies.

SUMMARY

1. A practical clinical method is presented for the quantitative estimation of acetone and acetoacetic acid in urine.

2. The method is based on the condensation of acetone and ortho-nitrobenzaldehyde in the presence of sodium or potassium hydroxide to give indigo.

3. The method is simple and accurate for the estimation of acetone and acetoacetic acid when these substances are present in the urine to a pathologic degree.

REFERENCES

1. Behre, J. A.: A Rapid Quantitative Method for the Determination of Acetone and Diacetic Acid in Urine, J. Lab. & Clin. Med. **13**: 1155, 1928.
2. Behre, J. A., and Benedict, S. R.: A Colorimetric Method for the Determination of Acetone Bodies in Blood and Urine, J. Biol. Chem. **70**: 487, 1926.
3. Deniges, G.: Sur les functions organiques pouvant se combiner au sulfate mercurique. Cas des acétones, Compt. rend. Acad. d. sc. **126**: 1868, 1898.
4. Dengies, G.: Combinaison, recherche, et dosage de l'acétone ordinaire avec le sulfate mercurique, Compt. rend. Acad. d. sc. **127**: 963, 1898.
5. Hubbard, R. S.: Determination of the Acetone Bodies in Urine, J. Biol. Chem. **49**: 357, 1921.
6. Van Slyke, D. D.: Studies in Acidosis. VII. The Determination of Beta-hydroxybutyric acid, Acetoacetic Acid, and Acetone in Urine, J. Biol. Chem. **32**: 455, 1917.
7. Van Slyke, D. D., and Fitz, R.: Studies in Acidosis. VIII. The Determination of Beta-hydroxybutyric Acid, Acetoacetic Acid, and Acetone in Blood, J. Biol. Chem. **32**: 495, 1917; **39**: 23, 1919.
8. Osterberg, A. E., and Helmholz, H. F.: Determination Whether Ketonurine Has Bactericidal Action, J. A. M. A. **102**: 1831, 1934.
9. German patent 19768 (1882).

A RAPID METHOD FOR THE ESTIMATION OF URINE SUGAR*

MICHAEL SOMOGYI, PH.D., ST. LOUIS, MO.

THE most satisfactory clinical approach for studying the carbohydrate utilization (tolerance) of diabetic patients is the one employed by Naunyn and his school at the turn of the century. Essentially it consists in preparing daily balance sheets of carbohydrate consumption and loss of glucose in the urine, the balance representing the amount of carbohydrate utilized in twenty-four hours.

In clinical and laboratory investigations during the past six years we have used this procedure routinely. In cases in which insulin therapy was applied, we did not stop at estimating the total glucose output for twenty-four-hour periods, but found it necessary to run separate quantitative determinations on every batch of urine collected between insulin injections; the information thus obtained proved to be the best criterion for the adequacy of the insulin dosage. One of the advantages of this procedure is that it can be pursued for any length of time without hardship to the patient, whereas gathering of the equivalent information by serial blood sugar determinations is scarcely tolerable for days and nights in a row.

The large number of sugar determinations in our laboratory (150 to 200 on some days) was feasible only with the aid of a rapid and simple method. We describe herein a procedure that we have been using during the past five years. It requires no more time and skill than a properly performed qualitative test.

The basis of our method is the well-known change of color which takes place when sugars are heated in alkaline solution; the shade which develops in the reaction varies from pale yellow to dark amber, depending upon the concentration of sugar. Our primary task was to devise experimental conditions under which the colors developed would be in the closest possible proportionality to the concentration of the sugar. As additional conditions we stipulated a reasonably short reaction period and sufficient sensitivity to permit the estimation of glucose over a wide range of concentrations.

Without enlarging on details of the exploratory experiments, let it be stated in brief that sodium carbonate as the alkali proved to satisfy the requirements. Glucose quantities varying from 1 to 30 mg. in 5 to 6 c.c. of reaction mixture yielded maximum color intensities when the concentration of the carbonate varied within the wide range of 8 to 15 per cent. The maximum color appeared after heating alkaline glucose solutions in a boiling water bath for seven minutes; prolongation of the heating beyond ten minutes caused a gradual fading of the color. (Boiling over an open flame for one and one-half to two minutes produces

*From the Laboratory of the Jewish Hospital, St. Louis.
Received for publication, November 29, 1940.

the same colors as heating in the water bath for seven to ten minutes.) In the procedure based upon these facts the following reagents and equipment are required:

Reagents.—

1. A 10 per cent solution of anhydrous sodium carbonate.
2. A series of standard glucose solutions, ranging in concentration from 0.5 to 6 per cent.

The standard glucose solutions are best prepared from a stock solution, a 10 per cent solution of glucose in water, saturated with benzoic acid (0.25 per cent) as a preservative. From the stock a series of 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 per cent standard solutions is prepared by dilution with water containing 0.25 per cent benzoic acid. Stock solution and standards keep indefinitely at room temperature.

Equipment.—

1. Test tubes of 14 mm. inside diameter. All test tubes employed must be of identical diameter. A simple way to select uniform test tubes is to measure accurately 10 or 15 c.c. portions of water into a series of tubes and to retain for use only those in which the water level is of the same height.
2. Pipettes for measuring 0.5 c.c. and 5.0 c.c. portions of fluid.

Procedure.—

Introduce into seven test tubes 0.5 c.c. portions of the standard glucose solutions, and into the other test tubes 0.5 c.c. portions of the urines to be analyzed, then add to every test tube 5 c.c. portions of the sodium carbonate reagent. Mix the contents, place the test tubes in a rack, and heat, immersed in boiling water, for eight minutes. Racks accommodating from one to two dozen (or more) test tubes are serviceable.

After eight minutes' heating read the results without undue delay. To this end place the standards in an ordinary test tube rack, leaving one space between each two tubes free for the matching of the test tubes containing the urine samples. When matching colors, view the test tubes transversely, holding the rack against a window or an artificial source of light. If the color of the unknown does not closely match any one of the standards, but lies between two of them, estimate the intermediate value by interpolation. If, for example, the color shade of an unknown is halfway between the 3.0 and 4.0 per cent standards, its sugar content is 3.5 per cent; if it is between 3.0 and 4.0 per cent, but distinctly nearer the 3.0 per cent standard, then it contains from 3.2 to 3.3 per cent of glucose, etc.

In laboratories where urine sugars are determined throughout the day, the slow but measurable fading of the colors at room temperature constitutes a drawback. One can get along with standards prepared once a day, but with the realization of the fact that analyses performed when the standards are several hours old will give readings that are 10 to 15 per cent too high. This is especially true on hot summer days, when the fading of colors is accelerated by high room temperatures.

Such degree of accuracy seems quite acceptable for a semiquantitative method; but we succeeded in eliminating this source of error by devising permanent standards, in sealed test tubes, which remain unchanged indefinitely. After an extensive search for adequate permanent standards, alcoholic iodine solutions were finally found best suited for the purpose; Dr. Simon Russi and other workers in our laboratory gave valuable assistance in this endeavor.

The stock solution for permanent standards is a 0.01 normal iodine solution in absolute alcohol from which a series of solutions is prepared by dilution with absolute alcohol. The normality of the several diluted solutions is given in Table I. A 0.008 normal alcoholic iodine solution is of the same color, shade, and intensity as the 6 per cent glucose standard and thus takes the place of it; a 0.0058 normal iodine solution is a substitute for a 5 per cent glucose standard, and so on, as given in Table I.

TABLE I

CONCENTRATIONS OF ALCOHOLIC IODINE SOLUTIONS USED AS PERMANENT STANDARDS

NORMALITY OF IODINE SOLUTIONS	CORRESPONDING PERCENTUAL GLUCOSE CONCENTRATIONS
0.0004	0.5
0.0009	1.0
0.0018	2.0
0.0028	3.0
0.0043	4.0
0.0058	5.0
0.0080	6.0

TABLE II

COMPARISON OF RESULTS OF URINARY SUGAR DETERMINATIONS BY THE NEW METHOD, WITH TRUE SUGAR VALUES ESTIMATED BY A COPPER-IODOMETRIC METHOD

NO.	GM. OF SUGAR PER 100 C.C. OF URINE	
	COPPER-IODOMETRIC METHOD, WITH SHAFFER-SOMOGYI REAGENT NO. 50	NEW METHOD
1	0.21	0
2	0.42	0.3
3	0.31	0.5
4	0.41	0.3
5	1.2	1.2
6	1.3	1.3
7	2.1	2.0
8	2.6	2.5
9	3.0	3.0
10	3.9	4.0
11	5.0	5.0
12	5.0	5.0

Approximately 5 c.c. portions of these iodine solutions are introduced into pyrex test tubes of 14 mm. inside bore and the tubes are then sealed. In this condition the standards remain unchanged without requiring any special precautions, as for example, protection from light or changes in room temperatures. The permanence can be readily ascertained by comparison with standards freshly prepared with pure glucose solutions. The earliest permanent standards in our laboratory are now over two years old and show no deterioration.

When using the permanent standards with a rather simple comparator block,* the accuracy of the method is great enough to advance it into the rank

*Sets of permanent standards with a matching block were placed on the market by A. S. Aloe Co., St. Louis, Mo., and are available through laboratory supply houses.

of adequate quantitative methods. The accuracy of this procedure is illustrated in Table II, in which results obtained by it are compared with determinations run by the copper-iodometric reagent of Shaffer and Somogyi.[1] It is to be remarked that the results of the copper-iodometric analysis represent true (fermentable) sugar values, that is to say, values that do not include any reducing matter other than sugar. These values, as may be seen, compare favorably with those obtained by the new procedure.

The method, we reiterate, is as rapid and simple as a properly executed qualitative test for urinary sugar, particularly when one is to carry out considerable numbers of determinations. In instances where only occasional single determinations are made, however, preliminary heating of a water bath to boiling and heating of the reaction mixture for eight minutes may involve a waste of time. This difficulty can be overcome by the use of an open flame. The analysis then is carried out as follows:

> Measure into a test tube 0.5 c.c. of urine and 5 c.c. of the carbonate reagent, drop in two small glass beads or quartz pebbles, and a drop of paraffin oil (or a corresponding bit of paraffin wax), heat to boiling over an open flame and, turning the flame low, keep the liquid *very gently* boiling for one and one-half to two minutes. The color develops to the same shade as after heating for eight minutes in a boiling water bath and can be matched against the permanent standards.

Since this method consumes no more time and work and requires no more skill than the generally employed qualitative methods, as for example Benedict's, in our institution the quantitative estimation of urine sugar has completely displaced the qualitative tests based upon copper reducing power.

A distinct advantage of the carbonate reaction is that it is more specific for sugars than are copper reagents. This quality comes mainly into play when dealing with concentrated urines, so frequent in hot summer weather, which may reduce appreciable amounts of copper without containing any abnormal amounts of glucose; with our method such urines give negative results.

If the urine is abnormally dark colored or contains blood or bile, it must be decolorized preliminary to the analysis. To this end about 2 to 3 Gm. (roughly one-half teaspoonful) of Lloyd's reagent is added to about 10 c.c. of urine in a test tube or a small flask, the mixture is gently agitated for about a minute (or longer) and then filtered through a dry filter paper. The nearly colorless filtrate is ready for analysis.

REFERENCE

1. Shaffer, P. A., and Somogyi, M.: J. Biol. Chem. **100**: 695, 1933.

MEDICAL ILLUSTRATION

LANTERN SLIDES: A NEW TECHNIQUE*

M. MUSCHAT, M.D., F.A.C.S., PHILADELPHIA, PA.

THE use of lantern slides in medical presentations is becoming more and more popular. The success of a presentation, whether long or short, depends largely upon the number and excellence of the slides shown. To the essayist it means a very definite aid in expounding and elucidating the details and fine points of the presentation. The audience is instantly aware and attentive, and remembers well the important part of the subject under discussion.

Our present-day photographic method of preparation of slides is rather uniform. Charts, graphs, tables, and drawings are photographed and positive slides are made. This method is good, but its cost is high and forces the average essayist to limit the number of slides he uses. By thus limiting himself to only a few slides he foregoes the opportunity of stressing many convincing points, leaving the impression of haste and incompleteness.

An additional method is with the use of cellophane, which is available as "announcement sheets" and "radio mats." The cellophane, encased in an envelope, is covered with a sheet of carbon paper. After the material is copied on the typewriter, the envelope is opened, the carbon copy is removed, and the finished film is placed between two cover glasses and taped. This method is simple and inexpensive, but it can only be used for writing a few sentences on a small area. Previous attempts to make charts or drawings on cellophane have ended in failure because of the small area (7.6 by 5.7 cm.) available, the smeary copy, and the inability to erase errors.

I am presenting here a novel method of slide preparation that is easy to handle and is low in cost. It consists of a very thin frosted paperlike film, upon which one can typewrite, draw with a pencil or pen and India ink, and even use water colors with good results. This material can be handled without difficulty, for it keeps its shape and does not become smeary. It possesses all the qualities and characteristics of a fine drawing or tracing paper. After the drawing or typing is completed, the frosted sheet is placed between two glass slides, bound with tape, and is ready for use. Thus an original drawing or chart can be prepared without having to resort to photography.

Material Used.—The frosted film is available on the market as "Traceolene" (light weight only), "Frosted Protectoids" (three weights or thicknesses:

*From the Departments of Urologic Surgery, Mount Sinai Hospital and the Medical School of the University of Pennsylvania, Philadelphia.

Received for publication, January 21, 1941.

light, medium and heavy), and "Plastacele" (varied thicknesses). In testing various thicknesses their optimum usefulness for specific purposes was found. The results of this study are summarized in Table I.

Typewriting.—The frosted film, cut into strips of 9.2 cm. width, is placed in the typewriter. The typing is done on the frosted side. It is better to use a few sheets of ordinary paper behind it, to obtain a sharper print. An extra black ribbon will produce darker print and show up better on the screen. The best print is obtained by placing the film in a folded carbon paper, thus obtaining a double print: front and back. One can utilize the entire surface area of the frosted film, leaving a margin of about 2 to 3 mm. for the binding tape (Fig. 1). The surface area thus available is 55 per cent greater than in the commercial cellophane method marketed as "radio mats." This ratio is shown in Fig. 2.

TABLE I

	LEAD AND CARBON PENCILS	INK	INDIA INK	STAMP	TYPE	PENCIL ERASURES	INDIA INK REMOVAL BY SCRATCHING	CLARITY OF TYPE	HANDLING
1 Cellophane	None	Poor	Poor	Poor	Fair	None	Poor	Smeary	Smeary
2 Traceolene or frosted: 88 AB	Poor	Poor	Good	Fair	Good	Good	Fair	Clear	Clean
3 Frosted: 150 AB	Good	Poor	Good	Fair	Good	Good	Good	Clear	Clean
4 Frosted: 300 AB	Poor	Poor	Good	Poor	Poor	Good	Good	Clear	Clean

Some projectors and lantern slide carriers are so constructed that they cut in on the sides of lantern slides in which the data extend too far to the right or left. It is advisable, therefore, to have the contents of the slide cover a space of only 7 cm. vertically and 7.5 cm. horizontally. This allows a space on the left of the image for attachment of a label or tab on which are noted the necessary data as to the owner, the nature of the contents, and the number of the slide. The large circle in the lower left-hand corner is an indicator for the projectionist to put the slide in the machine in the proper manner.

When a comparison is made of the print on clear cellophane with that on frosted film, the sharpness of the letter print in the frosted material and the smeary, unclean result on the cellophane can readily be seen. An enlargement of both results illustrates this difference in Fig. 3.

Plain rubber stamping can also be used, as seen in Fig. 4.

If partial legends are required, they should be printed with the proper spacing. The frosted paper is then cut to the correct sizes and placed on the drawing or directly on the slide as needed.

Drawing With Pencil.—In drawing an object on frosted film the same method is employed as when using ordinary paper. I recommend the use of carbon pencils since the marks left by these pencils will show up better on the screen. By using shaders dipped in carbon pencil dust on a sandpaper board, I was able to draw the most delicate shadings desired. Shaders are made by

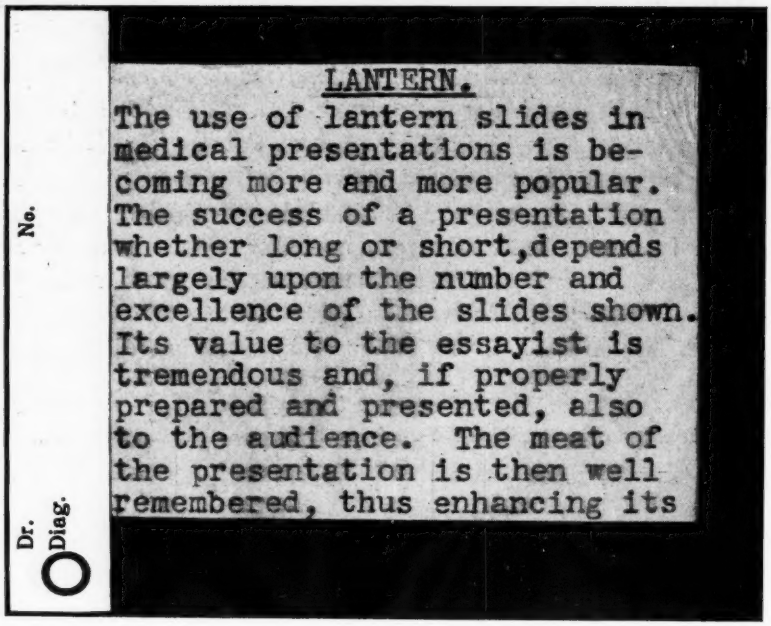

No.
Dr.
Dieg.
LANTERN.
The use of lantern slides in medical presentations is becoming more and more popular. The success of a presentation whether long or short, depends largely upon the number and excellence of the slides shown. Its value to the essayist is tremendous and, if properly prepared and presented, also to the audience. The meat of the presentation is then well remembered, thus enhancing its

Fig. 1.

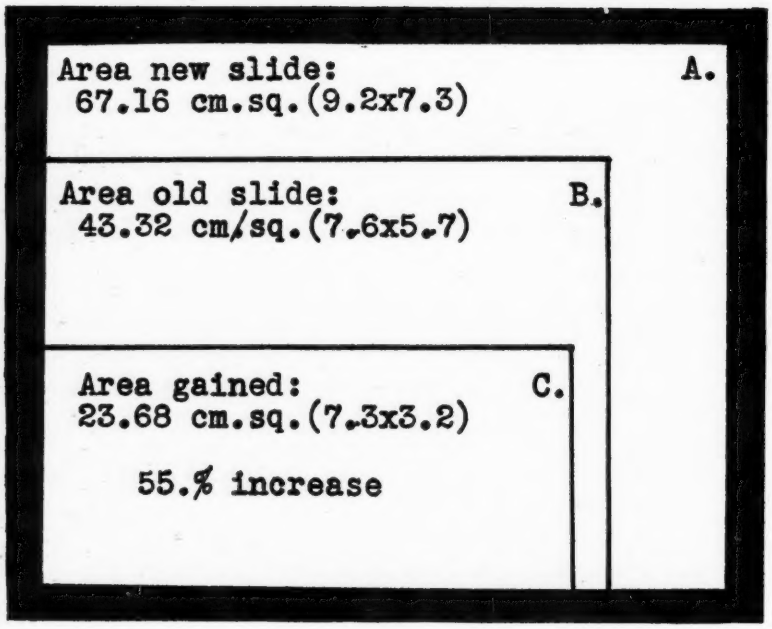

Area new slide:
67.16 cm.sq.(9.2x7.3)
A.
Area old slide:
43.32 cm/sq.(7.6x5.7)
B.
Area gained:
23.68 cm.sq.(7.3x3.2)
C.
55.% increase

Fig. 2.

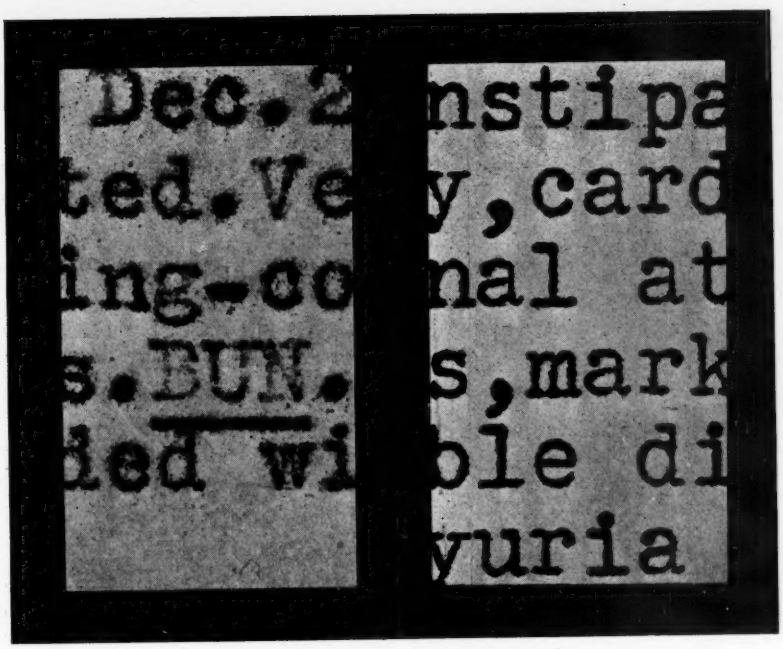


Fig. 3.

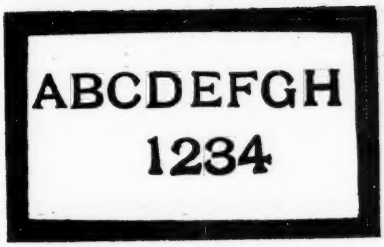


Fig. 4.

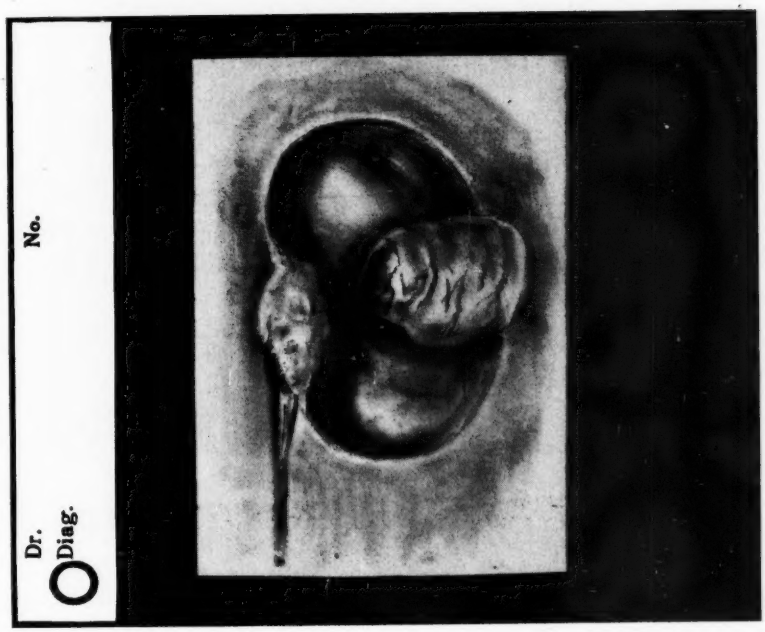


Fig. 5.

cutting and sandpapering to a point ordinary erasers and pieces of cork. A sharp, pointed pencil may be used on this frosted material without tearing or wrinkling. The film will stand erasing with hard and soft rubber (Fig. 5).

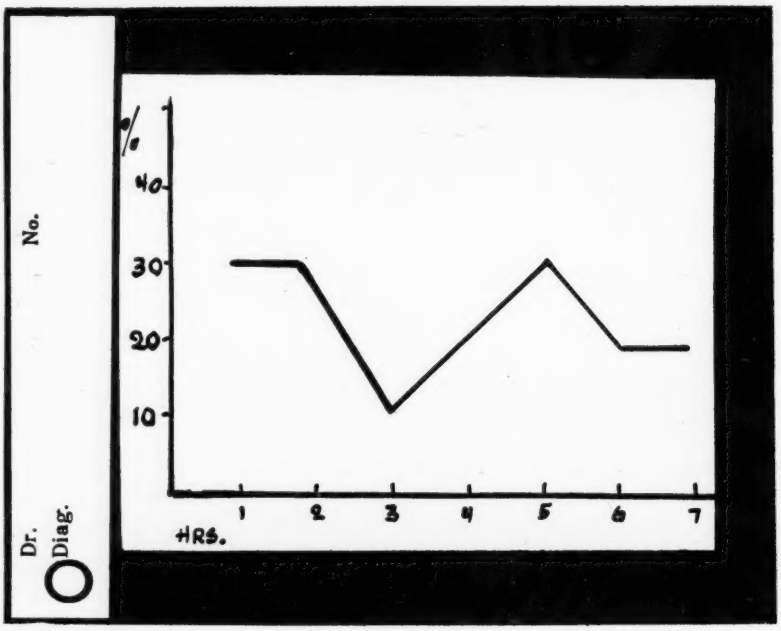


Fig. 6.

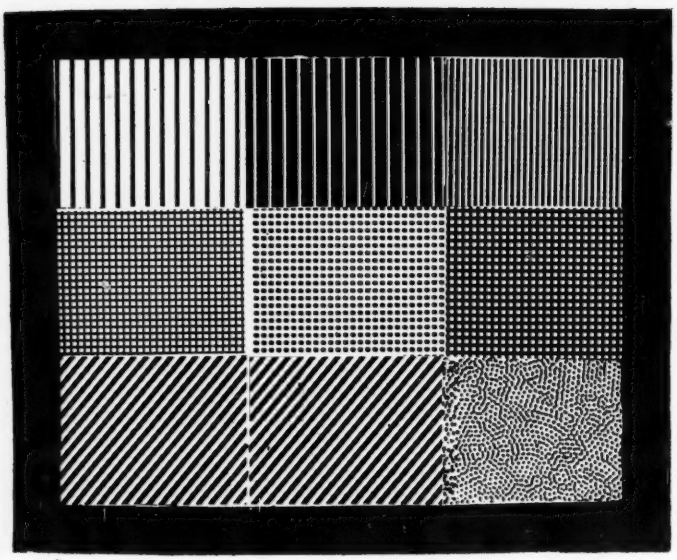


Fig. 7.

Drawing With India Ink.—The finest lines can be drawn on frosted film with pen and India ink. I drew charts and graphs on it the same as I do on fine drawing paper. Brilliant results may be achieved. Scratching out of the ink is easy. Ink smears on the glazed back may be wiped off with a wet sponge (Fig. 6).

Shading of Areas.—For this work I have utilized a special cellophane film with various designs or patterns of dots and lines printed on it. It is called "Transograph Shading Film"—A. Standard nonadhesive, and B. Contak-adhesive back (see Fig. 7). Spaces and columns were shaded by pasting them on the frosted film.

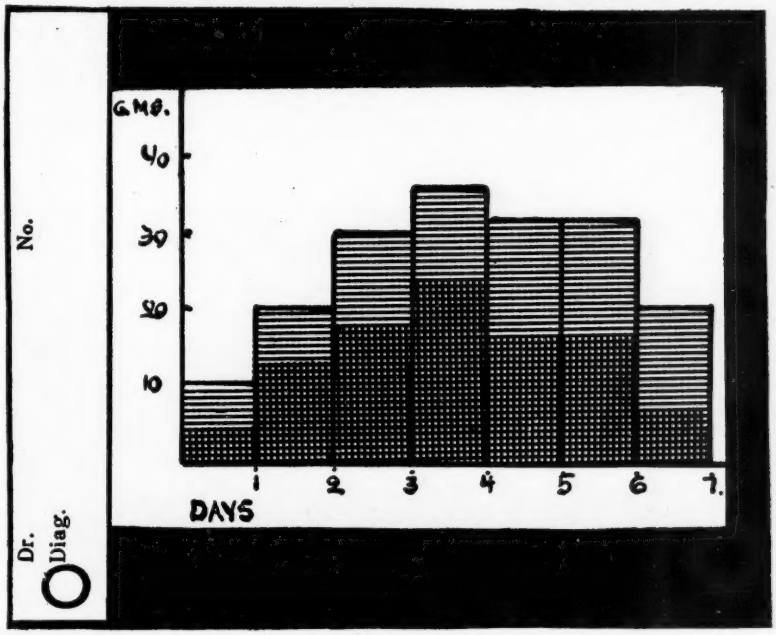


Fig. 8.

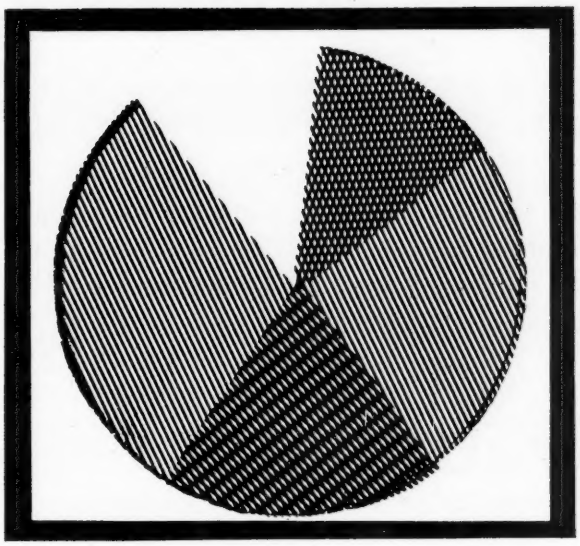


Fig. 9.

The shading film is useful in many ways. When using the Contak with the adhesive back, one side of the film is covered with a white paper upon which one

can draw with pencil or ink. After the required area is marked off, it is cut out. The protective white paper is then lifted off and the pattern is pasted directly on the frosted film. In order to produce varied shadings, the same film is pasted on the first layer of film, with the design running in a different direction. Many different types of shadings can thus be obtained. The handling is very simple and clean, and the final effect on the screen is brilliant (Figs. 8 and 9).

Tracings.—The frosted film is adaptable as a tracing paper in making a tracing of any desired image. By placing it upon the picture and fixing it with Scotch cellulose tape, one can trace any minute detail, either with carbon pencil or India ink. Such tracings can be very useful to those who cannot do freehand drawing.

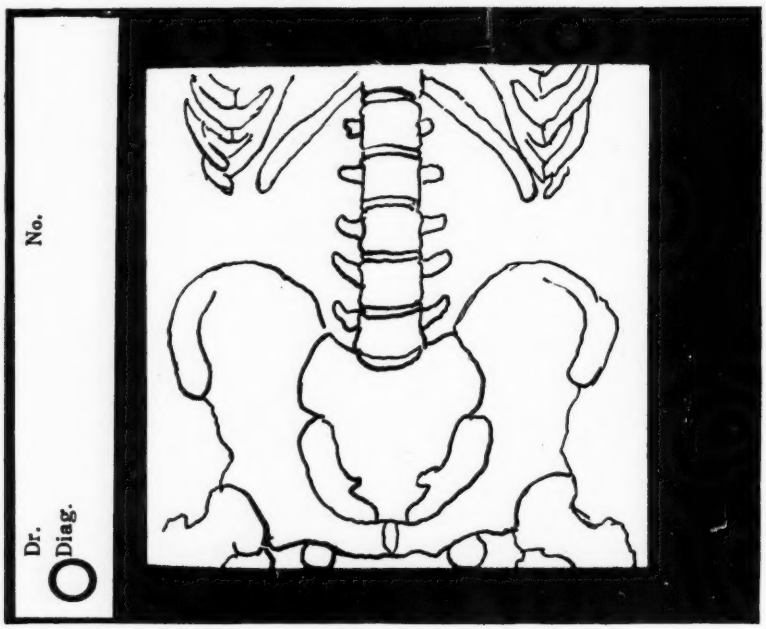


Fig. 10.

Color Slides.—Many desired effects in flat color can be obtained by the use of colored cellophane. The cut-to-shape colored cellophane, pasted on the frosted film, produces an effective color on the screen for differentiation. It can be pasted on the adhesive side of adherent cellophane, thus fixing it in permanent position. Colored cellophane is used for square and round graphs.

Permanency.—Slides made of frosted film are superior to thin cellophane in maintaining their shape and clarity over a long period of time. Under ordinary conditions they will not warp or wrinkle, and the type will not fade.

DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

SERUM LIPASE, Diagnostic Significance of Determinations of, Johnson, T. A., and Bockus, H. L. Arch. Int. Med. 66: 62, 1940.

This report comprises data on 616 independent determinations of serum lipase for 371 patients, performed over a period of three years. In the authors' experience, values for serum lipase are significant when they exceed 1 c.c. of a twentieth-normal solution of sodium hydroxide.

A large number of patients with functional and organic disorders, but with no clinical, surgical, or pathologic evidence of pancreatic disease were examined in order to determine the incidence of positive tests in nonpancreatic disorders. Uniformly normal lipase values were obtained in uncomplicated diabetes mellitus, hyperthyroidism, syphilis, tuberculosis, hypothyroidism, venereal lymphogranuloma, nonpancreatic malignant disease, uncomplicated peptic ulcer, gastritis, gastroduodenitis, functional colonopathies, nonobstructive organic diseases of the small and large intestines, and heart disease. The significance of normal values for lipase in these conditions is emphasized in order to stress the specificity of the test as an indication of pancreatic disease.

The study confirms the reports of others that diseases of the pancreas show the highest incidence of increased values for serum lipase. In 9 of 11 patients with acute pancreatitis and in 5 of 8 patients with proved cancer of the pancreas, pathologic values for lipase were obtained. Further proof that disease of the pancreas is the usual cause of elevations in serum lipase is supplied by the data for a group of 60 persons with cholelithiasis. A positive test was obtained in only 1 of 31 patients without jaundice and without a previous history of jaundice, whereas in 31 per cent of the remaining 29 patients with cholelithiasis with jaundice or with a history of jaundice values above 1.0 c.c. were obtained.

The study does not supply any support for the view that hepatocellular injury causes an elevation of the serum lipase. Normal values were obtained in 15 persons with so-called catarrhal jaundice, 2 with toxic or infectious hepatitis, and 12 with hyperthyroidism. These findings tend to throw some doubt on the theory that hepatic dysfunction may disturb the level of the serum lipase. If the findings in cases of catarrhal jaundice are confirmed by others, the lipase test may prove to be of distinct value in certain cases of jaundice of obscure causation, for an elevated value for serum lipase would tend to exclude the diagnosis of catarrhal jaundice.

As might have been anticipated, the authors noted no relation between hyperbilirubinemia and the values for serum lipase. This statement is based on simultaneous determinations of serum lipase and bilirubin on the same blood samples from 44 jaundiced patients. The fact may be considered additional evidence against the theory of a hepatic causation for hyperlipasemia.

In chronic hepatic disease associated with widespread destruction of the hepatic parenchyma, there was a rather high incidence of hyperlipasemia. High values were obtained in 7 of 24 patients with cirrhosis of the liver and in 2 with hepatic tumor. An examination of the material (Table 4) does not supply very convincing evidence that the possibility of pancreatic involvement was satisfactorily eliminated. The pancreas was examined at autopsy in only one patient (9) and was reported to be normal.

Four patients with intestinal obstruction had elevated values for serum lipase. The authors have found no reports of a similar observation in the literature. If the observation is confirmed by others, the serum lipase test will have another clinical application of importance.

The authors feel that this study justifies the conclusion that the serum lipase determination is of considerable clinical importance and that a wider application is warranted than it now enjoys.

SEPTICEMIA, Staphylococcic, Sutherland, R. T. Arch. Int. Med. 66: 1, 1940.

The treatment of staphylococcic septicemia should not be considered hopeless, since by early active treatment the mortality can be reduced.

Patients having active or recently healed carbuncles, furuncles, or blisters, with either prolonged, mounting, or septic temperatures for which no cause can be found; patients with symptoms and signs of a beginning osteomyelitis; and patients with staphylococci in the blood, should immediately have heroic treatment.

At present there is no standard as to what constitutes such treatment.

Experience suggests that in addition to supportive and surgical care, early treatment with large doses of antitoxin in a total dosage, varying from 300,000 to 1,000,000 or more units, together with some bactericidal element such as immune blood, gives encouraging results.

SCHICK REACTION, Effect of Human Serum on the Blood Antitoxin Titer and, Barenberg, L. H., Greenstein, N. M., and Leighton, B. Am. J. Dis. Child. 60: 36, 1940.

Because of the fact that susceptibility of a person to diphtheria can be determined within twenty-four hours by means of the Schick test and blood titration, diphtheria was singled out from other contagious diseases for special study to determine the mechanism by which the routine injection of human serum prevents the development of secondary contagious diseases in a hospital ward.

This investigation is based on observations of the effect of human serum on the cutaneous reaction in 141 children with positive reactions to the Schick test and on the blood titer of 55 of this group. The children ranged in age from 6 months to 12 years. The Schick test was performed on every patient admitted to the hospital, and a retest was given within several days after the serum injection. Subsequent retests, at monthly intervals, were carried out on patients in whom a reversal of the reaction followed serum injection. Intramuscular injection of from 10 to 30 c.c. of human serum was given to every patient within twenty-four hours after admission. Within twenty-four hours after the serum injection a central pallor developed in the area of the Schick reaction, and within forty-eight hours there was a clear demarcation between the central pallor and the peripheral inflammatory zone. In patients to whom serum was not given, this phenomenon did not occur. This anomaly did not indicate that a subsequent reversal of the Schick reaction would take place.

Injection of human serum brought about a reversal of the Schick reaction in 98 children, or 69.6 per cent. In 43 children, or 29.4 per cent, the Schick reaction remained persistently positive despite the serum injection. The intensity of the Schick reaction, however, apparently was reduced by means of the human serum. The modification of the reaction of 8 children who received from 10 to 15 c.c. was similar to that of 24 children who received from 20 to 45 c.c. of serum.

Titration studies were done on 55 children. Of 33 children in whose Schick reaction the serum brought about a reversal there was a significant rise in titer in 91 per cent, while of 22 whose Schick reaction was persistently positive there was only a slight rise in titer in 40 per cent. There was a close correlation between the rise in serum titer and the reversal of the positive Schick reaction. Analysis is presented of the observations on 39 children of the youngest age group, in whom 22 whose Schick reaction became negative after injection of serum had a rise in titer of 166.6 per cent, as compared with a rise of 44.4 per cent in 17 children whose Schick reaction was persistently positive. The average titer of the donors' serum in the former group was 0.0047 unit per cubic centimeter higher than the amount of the serum given to the latter group. These observations would indicate that the higher

potency of the serum was responsible for the reversal of the Schick reaction as well as for the rise in titer of the patients' blood. In this study the repetition of the Schick test was not responsible for the rise in the blood titer or for the reversal of the reaction, as the interval between the second test and the subsequent titration ranged between seven and thirty-three hours, a period in which the Schick material could not be a factor.

Human serum protected more than half of the children for twenty and five-tenths weeks and the remainder for three and two-tenths weeks. The average period of protection was thirteen weeks in the cases of the youngest group of children, as compared with twenty-two and twenty-six weeks in those of the two older groups. It was found that the youngest age group, in which the smallest percentage was protected, lost its immunity sooner.

MONONUCLEOSIS, Infectious, The Histological Lesion in Lymph Nodes in, Gall, E. A., and Stout, H. A. Am. J. Path. **16**: 95, 1940.

A survey of the literature reveals the fact that no consistent lesion has hitherto been described in the lymph nodes from patients with infectious mononucleosis. The authors have studied lymph nodes removed from ten such patients at various stages in the illness and have described a characteristic morphologic pattern. This appears with such regularity in this disease and so rarely in other conditions that it is believed to have diagnostic importance.

The basic lesion of infectious mononucleosis is apparently the result of the varied responses of several different elements composing the lymph node to a single, presumably irritative stimulus. The structures manifesting this reaction may be enumerated as follows: (a) lymphoid follicles, (b) lymphoid cords, (c) lymph sinuses, (d) sustentative elements and blood vessels.

The important crude diagnostic feature which serves in the differentiation from primary neoplastic disease of the lymph node is the retention of gross architectural relationships. This is particularly the case with reference to the persistence of subcapsular and radial sinuses.

The basic process underlying the lymph node lesion in this disease is essentially the result of proliferative stimulation of the components of the node.

In what appears to be the early stages of the lesion, the germinal centers of the lymph follicles become hyperplastic and show large secondary nodules. These consist of masses of apparently fused cells with abundant, poorly defined, basophilic cytoplasm and large vesicular nuclei (stem cells), and also of varied numbers of mononuclear elements with more sharply defined and demarcated eosinophilic cytoplasm and eccentrically placed lobulated or reniform nuclei (probably clasmatocytes). Mitotic figures are numerous and there is evidence of phagocytic propensity among many of the clasmatocytes.

At the same time there are increased numbers of mitotic figures in the larger cells of the extrafollicular lymphoid substance in both cortical and medullary regions.

During the florid stage of the disease the appearance of the lymph node is quite unusual and presumably specific. The node is enlarged and shows greatly increased cellularity in the medullary, cortical, and sinus substance. The cords are swollen by a rich mixture of small and large lymphocytes, stem cells, and lymphoblasts, infectious mononucleosis cells, and large eosinophilic phagocytic elements. Follicles persist in some cells, but in others they are apparent only as occasional, partially disrupted germinal center fragments, and reticulum stains exhibit a vestige of concentric perifollicular arrangement. There is marked sinus compression and distortion, although identity is preserved. The sinus lumina contain variable numbers of cells similar to those noted in the pulp. Cells of this type are also evident in small numbers in both the trabeculae and capsule of the node. There is an apparent increase in reticulum meshwork fibrils, vascular channels are much more abundant than usual, and vascular endothelium is hyperplastic.

BILIRUBIN, Comparison of Tests for, in Urine, Foord, A. G., and Baisinger, C. F. Am. J. Clin. Path. **10:** 238, 1940.

In doing tests for bilirubin in the urine, particularly to avoid missing small quantities, some method of concentration of the pigment should be used, either precipitation and adsorption by barium chloride or adsorption by talc. The most commonly used tests on unconcentrated specimens are not sensitive and often are difficult to read.

The diazo spot method of Godfried is a highly satisfactory method, as are also the Harrison spot and the Naumann methods. The latter is perhaps more time-consuming. The red color developed in the diazo spot method is more easily read than the colors produced by the other methods.

Diazo Spot (Godfried).—Precipitate bilirubin with barium chloride, filter, spread filter paper on dry filter paper, and add 1 drop of diazo reagent, 4 drops of 95 per cent alcohol, and 1 drop of phosphate buffer. Red to pink color denotes a positive reaction. Test works best with small amounts of bilirubin. If precipitate is deep yellow in color, add more of reagents, or better, dilute urine and repeat. A quantitative technique by diazo method is given by this author.

Harrison Spot (Godfried).—Add to 10 c.c. urine 5 c.c. of 10 per cent barium chloride solution, mix and filter. Spread filter paper on dry filter paper. Add 1 to 2 drops of Fouchet's reagent (trichloracetic acid 25 Gm., water 100 c.c., 10 per cent ferric chloride 10 c.c.). Blue to green color indicates a positive reaction.

Naumann.—Filter through paper in a Buchner funnel of 3.5 cm. diameter 5 c.c. of a 10 per cent emulsion of talc in water. Suck nearly dry. Add 5 c.c. of urine and suck nearly dry. Add 1 drop of Fouchet's reagent or 10 per cent nitric acid in middle of disk. Blue color is positive.

BONE MARROW, Sternal, in Hyperthyroid and Myxedematous States, Jones, R. M. Am. J. M. Sc. **200:** 211, 1940.

Sternal marrow aspirated from 18 "normal" individuals contained an average of 6.2 per cent nucleated cells.

Sternal marrow from 12 individuals with hyperthyroidism contained an average of 13.5 per cent nucleated cells, about 2⅕ (217 per cent) times the "normal" finding.

Sternal marrow from 7 individuals with hypothyroidism contained an average of 2.4 per cent nucleated cells, a little more than one-third (38 per cent) of the "normal" average.

Five patients with hypothyroidism treated with desiccated thyroid or thyroxin, and subsequently studied, showed a marked rise in the percentage of nucleated cells in the sternal marrow.

A patient with thyrotoxicosis following thyroid ingestion showed a marked decrease in the percentage of nucleated cells in the marrow when the thyroid was stopped. There was a less marked but definite decrease in patients where subtotal thyroidectomy was performed.

The hyperplasia found in the marrow of the hyperthyroid individuals was myeloid in character, and was not reflected in the peripheral blood.

SULFANILAMIDE, Effect of Sulfapyridine and, Upon the Blood Pigments of White Rats, Smith, P. K. Am. J. M. Sc. **200:** 183, 1940.

In white rats that had received orally for one month daily doses of sulfanilamide, 700 mg. per kilogram, small, but significant, amounts of methemoglobin and somewhat larger amounts of sulfhemoglobin were found. By comparison with a group of control rats there was a decrease in total hemoglobin of about 10 per cent.

Under similar conditions a daily dose of sulfapyridine, 1,050 mg. per kilogram resulted in a similar amount of methemoglobin without an appreciable amount of sulfhemoglobin. There was no significant reduction in total hemoglobin.

PERNICIOUS ANEMIA, The Erythrocyte Response to Treatment, Riddle, M. C. Am. J. M. Sc. **200**: 146, 1940.

From data in 523 patients with pernicious anemia satisfactorily treated, the average weekly increase in the erythrocyte count at the end of two weeks of treatment was found to bear an inverse relationship to the erythrocyte count before treatment.

This relationship is expressed in the equation $I = 0.78 - 0.174\ Eo$, where *I* is the average weekly increase in the erythrocyte count after two weeks of treatment and *Eo* is the erythrocyte count before treatment, expressed as millions of erythrocytes per cubic millimeter of blood.

This equation is suggested as a standard for measuring the relative effectiveness of treatment in pernicious anemia.

Observed values equal to or greater than those obtained from this equation indicate adequate treatment. Observed values less than those calculated from the equation indicate inadequate treatment.

The existence of various complicating factors such as concurrent disease, transfusion, or hemorrhage in association with pernicious anemia, invalidate the use of this standard.

HEMOLYTIC ANEMIA, Erythrocyte Morphology in Experimental as Induced by Specific Hemolysin, Tigertt, W. D., Duncan, C. N., and Hight, A. J. Am. J. M. Sc. **200**: 173, 1940.

The administration of a specific hemolysin to the donor animal (dog) is followed by a fall in the erythrocyte and hemoglobin levels, proportional to the amount of hemolysin administered and to the titer of the hemolysin.

In the process of cell destruction due to hemolysin (in contrast to the mechanism of hypotonic hemolysis), the erythrocytes approach a spherical form by a diminution in diameter associated with little or no increase in corpuscular volume.

This approach to sphericity is paralleled by proportionately decreased resistance to hypotonic hemolysis.

The length of life in the blood stream of the erythrocytes altered by the hemolysin is inversely proportional to the amount and titer of the hemolysin administered, and survival periods up to one month have been observed.

CEREBROSPINAL FLUID, Calcium and Phosphorus in, in Diabetes Insipidus, Blotner, H. Am. J. M. Sc. **200**: 235, 1940.

The concentrations of the calcium, phosphorus, chlorides, and protein were studied in the spinal fluid and blood serum in 10 patients with diabetes insipidus.

The average concentration of the spinal fluid calcium was slightly greater than that of the Ca^{++} content of the serum (1.51 vs. 1.27 mM per liter) in these patients. A series of control patients showed no such difference between their serum Ca^{++} concentrations and cerebrospinal fluid calcium (1.27 vs. 1.24 mM per liter).

Analyses for inorganic phosphate, chloride, and protein showed no significant changes.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, 201 West Franklin Street, Richmond, Va.

Cellula*

CELLULA is the first volume of a four-volume set to be published over the years 1939 to 1941, and will cover the whole field of cytology. The contents consist mainly of figures with very little text. Figures in the widest sense of the word are included, e.g., absolute figures, metrical and dynamic constants, formulas and curves, all data of exact biology for basing scientific conceptions on exact measures.

In this first part there are four sections: Biometry of the animal and plant cell (G. Teissier, Paris); Tissue cell colonies in vitro (E. Mayer, Beirut); The colloidal properties of the cell (J. S. Mitchell, Cambridge); The division of single cells (F. Buchthal and G. G. Knappeis, Copenhagen).

Selected by experts, these data represent a gold mine of information.

Syphilis†

THERE are many ideas by the author which are at variance with those generally held by the medical profession. Thus he is of the opinion that syphilis is incurable and as a consequence that syphilitics should all be sterilized and forced to marry other syphilitics or not at all. His conclusion is that syphilis is "a disease, the very name of which is prudishly avoided by the lay press, and the victims of which society hates and condemns, the state prosecutes and punishes, and the medical profession deceives and plunders, and which is impossible to fight in the open, cannot be defeated, conquered and subsequently exterminated." The book is more literary than scientific.

Leprosy‡

LEPROSY, one of the most ancient of diseases, now bids fair to being controlled. The chief milestones in this achievement were the discovery of the etiologic agent by Hansen and the new improved treatment with chaulmoogra oil. Thus the former main reliance on lifelong compulsory isolation in prophylaxis has been nearly universally modified; nonfectious or only slightly infectious cases can now be treated at outpatient clinics.

Several points brought out in the book are of special interest to Americans. There is an endemic focus in Louisiana and a second in the North Central States, though the latter seems to be disappearing. The national leprosarium at Carville is said to have been developed into the best equipped hospital and colony in the world, which should in time effect the desired reduction of the disease in the United States. The total number of cases in this country is estimated at 1,865, or 0.02 per mille.

No phase of the subject is neglected in this splendid monograph, which after a lapse of fifteen years has been revised. Written by two internationally famous leprologists, the book may be regarded as completely authoritative.

*Cellula. Edited by Hans Handovsky, Professor of Pharmacology, Now "Chef de travaux" in the Pharmacological Department of the University of Ghent. Part I. G. Teissier (Paris); Biometrie de la cellule animale et vegetale; Edmund Mayer (Beirut); Tissue cell colonies in vitro; J. S. Mitchell (Cambridge): The colloidal properties of the cell; F. Buchthal and G. G. Knappeis (Copenhagen): Die Doppelbrechung der einzelnen Zelle. Paper, 374 pages, W. Junk, The Hague, 1939.

†Syphilis and Its Accomplices in Mischief: Society, The State and the Physician. By George M. Katsainos, M.D. Paper, 676 pages. Privately printed at Athens, Greece.

‡Leprosy. By Sir Leonard Rogers, K.C.S.I., C.I.E., M.D., F.R.C.P., F.R.C.S., F.R.S., I.M.S. (Retired), Late Physician and Lecturer, London School of Tropical Medicine, and Professor of Pathology, Calcutta Medical College; and Ernest Muir, C.I.E., M.D., F.R.C.S., Edinburgh, Medical Secretary, British Empire Leprosy Relief Association, Late Research Worker in Leprosy at the School of Tropical Medicine and Hygiene, Calcutta. Cloth, ed. 2, 260 pages, $4.50. A William Wood Book. The Williams & Wilkins Company, Baltimore, 1940.

Simplified Diabetic Manual*

THE chief value of this excellent manual appears in the second part which deals with dietetics in a very lucid manner. Complete, and it may be said, understandable directions are given for altering the menu without changing the formula. A large number of international recipes are included in the book, with perhaps greater emphasis being put on Jewish dishes.

Extensive tables of food values and mineral and vitamin contents are included.

It must not be inferred that the requisite information needed by the diabetic person is withheld, but the manual does not induce the patient to self-treatment.

Physicians may confidently recommend this book to their diabetic patients.

Handbook of Public Health Bacteriology and Chemistry†

ALTHOUGH small, the manual is well suited to its aim of coordination of the work of the practitioner with that of the public health department. Attention is called to several basic errors in the use of laboratory service: those arising from the use of improper specimens or inadequate information submitted with the specimens; from administration by regulation rather than judgment; and finally from interpretation of laboratory results. There is a résumé listing the materials to be collected and the laboratory procedures which are pertinent for all the infectious and communicable diseases of California. Information is also given as to the best methods of shipment of specimens.

Physiology of Micturition‡

AFTER giving a description of the anatomy of the urinary bladder and urethra, the authors turn to a review of the drugs dealing with the vesical muscles. Urgency and frequency of urination are relieved by atropine, and further improvement is obtained by giving ephedrine at the same time. The new and more stable compounds of acetylcholine will stimulate strong vesical contractions.

The act of micturition is described next. Urination can only be initiated by a contraction of the vesical muscle itself. The vesical orifice does not open voluntarily, but following contraction of the detrusor. It is possible to close the sphincter. The vesical muscle is extremely sensitive to intellectual and emotional responses.

Bladder symptoms in tabe dorsalis, in lesions of the cauda equina, in spina bifida after cortical injury, and in Parkinsonian syndrome, are carefully discussed.

Clinical Methods§

OF CONVENIENT pocket size this handbook contains everything the student requires when he begins work on a medical ward. Directions for history taking are followed by descriptions of techniques in physical diagnosis and in laboratory tests.

---

*Simplified Diabetic Manual With 163 International Recipes (American, Jewish; French, German, Italian, Armenian, etc.). By Abraham Rudy, M.D., Associate Physician and Chief of the Diabetic Clinic, Beth Israel Hospital, Boston, Instructor in Medicine, Tufts College Medical School, Consultant in Diabetes, Jewish Memorial Hospital, Roxbury, Mass., and Jewish Tuberculosis Sanatorium, Rutland, Mass. With an Introduction by Dr. Frederick M. Allen. Cloth, 216 pages, $2.00. M. Barrows & Company, Inc., New York, 1940.

†Handbook of Public Health Bacteriology and Chemistry. General information regarding epidemiology, collection, and shipment of specimens, and bacteriologic, serologic, and chemical procedures. Edited by M. S. Marshall, Department of Public Health, City and County of San Francisco, Calif., J. C. Geiger, Director. Paper, ed. 2, 150 pages, 1939.

‡Physiology of Micturition. Experimental and Clinical Studies With Suggestions as to Diagnosis and Treatment. By Orthello R. Langworthy, Lawrence C. Kolb, and Lloyd G. Lewis, Sub-Department of Neurology and James Buchanan Brady Urological Institute, The Johns Hopkins University. Cloth, 232 pages, $3.50. The Williams & Wilkins Company, Baltimore, 1940.

§Clinical Methods. A Guide to the Practical Study of Medicine. By Sir Robert Hutchison, Bart., M.D., LL.D., F.R.C.P., Consulting Physician to the London Hospital and to the Hospital for Sick Children, Great Ormond Street, President of the Royal College of Physicians; and Donald Hunter, M.D., F.R.C.P., Physician to the London Hospital. Cloth, with 19 color, 8 halftone plates and 106 figures, 622 pages, $5.00. Paul B. Hoeber, Inc., Medical Book Department of Harper & Brothers, New York, 1940.

Although terse, the book is authoritative, having been written by two outstanding British physicians. It would appear that a text of this nature would also serve admirably for review for advanced medical examinations. The value set on this manual by the profession is attested to by the fact that it has been used successfully for over forty years and has passed through eleven editions. The latest issue is entirely up to date.

In the last section, on clinical diagnosis, the common types of neurologic disease giving rise to vesical abnormalities are discussed and typical examples are given. Many obscure cases of urinary disturbance are dependent on abnormalities of the brain and cord. Disseminated sclerosis ranks next to syphilis in this regard. A certain number of cases are functional. Cystometric methods are emphasized throughout the book. A very good bibliography and index are included.

This book must be read by all who are interested in the urinary bladder, and particularly by urologists and neurologists.

Multiple Human Births*

SEVENTEEN years ago Dr. Newman's book, the *Physiology of Twinning*, was reviewed in these pages. The present volume brings his studies up to date in a popular presentation. It is the first of a series of semipopular books on subjects of science to be published under the sponsorship of the American Association for the Advancement of Science.

Dr. Newman has been most successful in simplifying his presentation for the lay reader and in avoiding technical terminology. Thus, instead of referring to monozygotic twins, he consistently calls them one-egg twins.

With two million twins, triplets, and quadruplets in the United States, there must be ten million persons closely related to twins in one way or another. The volume should, therefore, be of interest to many.

Sections which should be of especial general interest include Siamese Twins, Mirror Imaging, the Dionne Quintuplets, Criminal Twins, and the Life Stories of One-egg Twins who have been reared apart, usually twins who have been left in orphanages. His stories of their chance meetings would be very appropriate for a "Believe It or Not" column.

The Association for the Advancement of Science is to be congratulated for having selected such a thoroughly interesting volume for the inauguration of the series.

The Englishman's Food†

DR. DRUMMOND, who is professor of biochemistry at University College, London, presents almost in novel form an analysis of the foods that the Englishman has eaten since medieval times. The volume represents a tremendous amount of historical research. It possesses a definite medical facet in that the authors analyze the probable food deficiencies in the various centuries, special emphasis being given to the vitamins.

Illustrative of the material covered, Part One deals with Medieval and Tudor England: Production of Food, Quality of Food, Meals of the People, and Their Diet and Health. Deficiency diseases are discussed. The last part, "The Twentieth Century" chapter, deals with dietary problems in the World War. In an Appendix the authors estimate the composition and food value of some of the diets discussed in various parts of the text.

The volume has illustrations gathered from the old English literature.

It may be recommended not only as a reference volume for all who are interested in the history of foods and in nutrition in general but also as a volume of very interesting general reading.

---

*Multiple Human Births. Twins, Triplets, Quadruplets and Quintuplets. By Horatio Hackett Newman, Ph.D., Sc.D., Professor of Zoology, University of Chicago. Cloth, 214 pages, $2.50. Doubleday, Doran & Company, Inc., New York, 1940.

†The Englishman's Food. A History of Five Centuries of English Diet by J. C. Drummond, Professor of Biochemistry, University College, London; and Anne Wilbraham. 574 pages. Jonathan Cape, Thirty Bedford Square, London. 1939.